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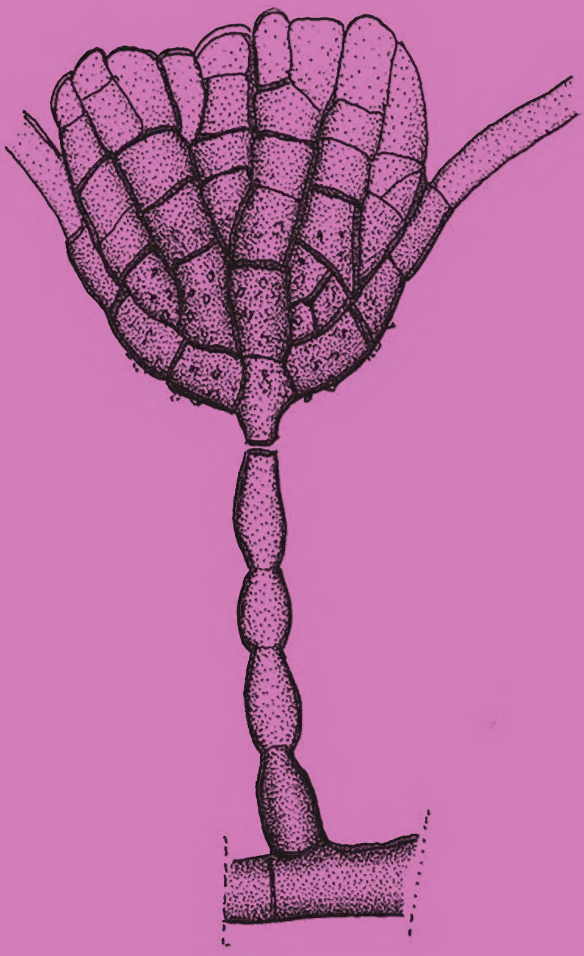

X. indica in *Xiuguozhangia* gen. nov.
(Kai Zhang & al.— FIG. 4, p. 133)
RAFAEL F. CASTAÑEDA-RUÍZ, *artist*

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Volume 127

Validation of the name *Diplococcium variegatum*

Silvana Santos da Silva, Alisson Cardoso Rodrigues da Cruz, Luís Fernando Pascholati Gusmão, & Rafael F. Castañeda-Ruiz

The proposed name "*Diplococcium variegatum* S.S. Silva, Gusmão & R.F. Castañeda, sp. nov." on page 60 of volume 127 was not validly published because the cited registration number, "MB 805030", was incorrect and is assigned to a separate name. Art. 42.1 of the International Code of Nomenclature (McNeill & al. 2012) requires that after January 1, 2013, 'the citation in the protologue of the identifier issued by a recognized repository for the name (Art. 42.3) is an additional requirement for valid publication.' We hereby validate the name *Diplococcium variegatum* S.S. Silva, Gusmão & R.F. Castañeda, sp. nov. with the assigned number MB 805072, with its description published in Mycotaxon 127: 60. 2014 and its holotype designated as HUEFS 194254. Additional details were published in the original publication.

Other Errata for Mycotaxon 127:

p. ii, between lines 11 & 12 Add: Karen Hansen (2014–2019)
Stockholm, Sweden

p.115, line 3 *for:* Milagro Granados[1]
read: Milagro Granados-Montero[1]

Reviewers — volume one hundred twenty-eight

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

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From the Editor-in-Chief

Karen Hansen joins the Mycotaxon Advisory — Through an unfortunate oversight, we did not list our new Editorial Advisory member in her rightful place on p. ii of Mycotaxon 127. Karen Hansen (Stockholm, Sweden) was elected in January to serve as advisor to the Editors during 2014–2019. We are lucky to have her and take this official opportunity to forward an appreciative welcome while apologizing profusely for our editorial negligence!

IMC accepts three nomenclatural repositories: On 8 August 2014, the Tenth International Mycological Congress resolved during its closing ceremonies in Bangkok to accept "the decision of its Nomenclature Session with respect to Art. 41 of the International Code of Nomenclature for algae, fungi, and plants, made 7 August 2014 regarding official repositories for the registration of fungal names, namely to recognize multiple repositories: Fungal Names, Index Fungorum, and Mycobank ...."

Correct numbers *required* for valid publication of new names — The first erratum on p. vi of this volume, slightly more elaborate than usual, points to the need for authors to scrutinize *carefully* their nomenclatural registration numbers before submitting a paper for publication. Because such numbers are hidden from external inspection until after publication, we — as editors — can not check them at our end. Remember that the difference of one digit will refer to a name that differs from what is intended and thereby invalidates the name. Each number is unique and must be rendered accurately or the name cannot be regarded as valid.

Mycotaxon 128 contains 28 papers by 94 authors (representing 14 countries) and revised by 59 expert reviewers. Within its pages are two new genera (*Anacraspedodidymum* —including a new species from Brazil— and *Xiuguozhangia*— honoring China's Xiu-Guo Zhang) and 16 other species new to science representing *Arachnophora, Atelocauda, Dactylaria,* and *Ravenelia* from Brazil; *Aureoboletus, Chaetospermum, Diacheopsis, Hymenochaete, Sphaceloma, Thalloloma,* and *Xylaria* from China; *Marasmius* and *Muscodor* from India; and *Tylopilus* from Pakistan. In addition to range extensions and/or new hosts for previously named taxa, five new names (in *Peziza* and *Puccinia*) and 6 new combinations (in *Anacraspedodidymum* and *Xiuguozhangia*) are proposed.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
12 August 2014

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Tylopilus: a new species and a new record from Pakistan

S. Sarwar*, A.N. Khalid, & A.R. Niazi

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Abstract — *Tylopilus sultanii* sp. nov. and *T. pseudoscaber* are reported from Pakistan, occurring in forests dominated by ectomycorrhizal trees of *Pinaceae*. The collections are described and illustrated, and their habitat is described. Their phylogenetic relationships were accessed by ITS sequence analysis. *Tylopilus pseudoscaber* represents a new record for Pakistan. A key to the three *Tylopilus* species recorded in Pakistan is presented.

Key words — *Basidiomycota*, biogeography, *Boletaceae*, conifers, Himalaya, phylogeny

Introduction

Khyber Pakhtunkhwa (KPK), Pakistan, which lies at an altitude of 1000–4000 m in the Himalayan range, is mainly dominated by conifer forests and regarded as one of the world's 35 biodiversity hotspots (Myers et al. 2000, Zachos & Christian 2011). The Mansehra, Dir, Swat, Malakand, and Abbottabad districts of KPK, and the Rawalpindi district of the Punjab are covered mainly by conifer forests (Anwar 2008), including fir (*Abies*), deodar (*Cedrus deodara*), Kail (*Pinus wallichiana*), and chir pine (*Pinus roxburghii*). Normal growth and survival of pinaceous trees depends on colonization by ectomycorrhizal (ECM) fungi (Smith & Read 1997).

The ectomycorrhizal genus *Tylopilus* P. Karst. has a rich biogeographical history (Wolfe & Bougher 1993, Halling 1996) and is characterized by smooth spores, a pinkish to black pore surface, solid stipe lacking an annulus, and glandular dots (Bessette et al. 2000). Seventy-six *Tylopilus* species are known worldwide (Kirk et al. 2008, Osmundson & Halling 2010), but molecular analysis shows that the genus is polyphyletic (Binder & Hibbett 2006).

Only one *Tylopilus* species, *T. felleus* (Bull.) P. Karst., has been reported from Pakistan (Razaq 2007). We describe here a new species, *T. sultanii,* and a new record for the country, *T. pseudoscaber*. A key to the three *Tylopilus* species in Pakistan is also provided.

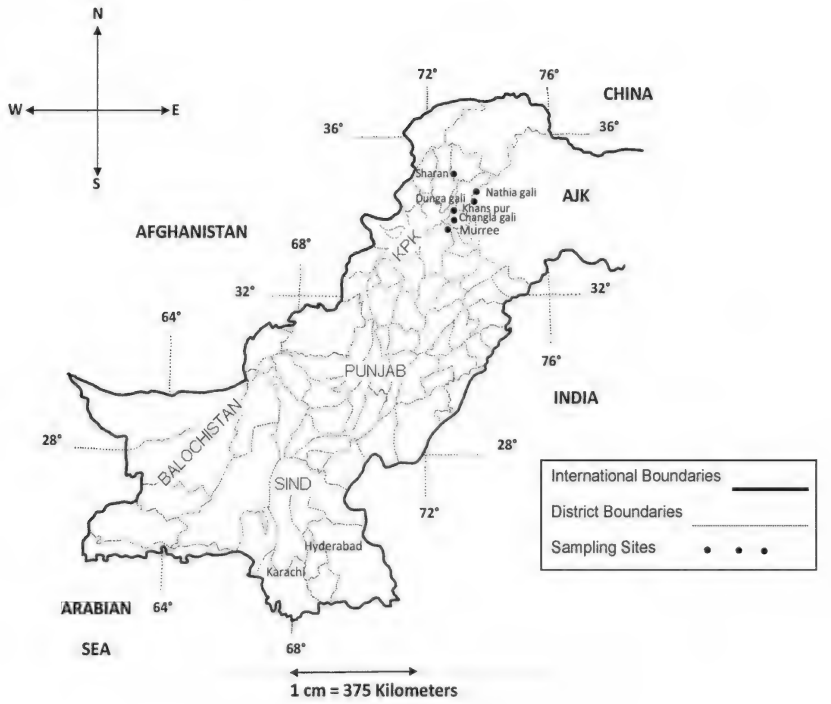

FIGURE 1. Map of Pakistan showing sampling sites.

Materials & methods

Sporocarp collection

Specimens were collected from sampling sites (FIG. 1) in early to late summer (July–September) during 2006, 2009, and 2011. Field notes and digital photographs were made from fresh specimens. Sporocarps were dried with hot air from a fan heater and then kept in paper bags for processing in the lab. Voucher specimens were deposited in the bolete collection of the Lahore Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Morphological characterization

Characters recorded from fresh sporocarps included:

PILEUS: Color, shape, diameter, texture and ornamentation, context color and color changes, margin shape and color.

HYMENOPHORE: young and mature tube and pore color, tube and pore size, and bruising reactions of the pore surface.

STIPE: Color, shape, diameter, texture and ornamentation, context color and color changes, presence/absence of annulus, attachment to the pileus.

Characters recorded after mounting dried sporocarp tissue in KOH, Meltzer's reagent, Trypan blue, and Lactic acid included:

> Shape, length, width and cytoplasmic contents of basidia, cystidia, basidiospores, pileipellis and terminal cells of pileipellis; color reaction of all these in KOH, Meltzer's, lactic acid and Trypan blue.

Molecular analysis

DNA was extracted from dried sporocarps by using enzymatic digestion and glass-fibre filtration (EDGF) protocol (Dentinger et al. 2010). The nuclear ribosomal internal transcribed spacer region (ITS) was amplified following the PCR conditions of Dentinger et al. (2010) using primers ITS3 (White et al. 1990) and ITS6R (Dentinger et al. 2010). PCR products were purified using ExoSAP-IT® (Affymetrix, High Wycombe, UK) and dye-terminated unidirectional sequencing was performed using a BigDye®Terminator v3.1Cycle Sequencing Kit (Life Technologies/ABI, California, USA) in 10μL reactions with primers ITS3 and ITS6R following the protocol of Dentinger et al. (2010). Sequencing reactions were cleaned using ethanol precipitation following the manufacturer's instructions, resuspended in 30 μL of distilled water, and run on an ABI 3730 DNA sequencer in the Jodrell Laboratory, Royal Botanic Gardens Kew. Sequence chromatograms were edited by comparing overlapping reads using BioEdit and comparisons to GenBank records using BLAST. Sequences have been deposited in a single file in GenBank (*Tylopilus pseudoscaber:* KJ775785; *T. sultanii:* KJ775786). The sequences were aligned using MUSCLE alignment software (Edgar 2004). Phylogenetic trees were constructed with the maximum likelihood algorithm and Jukes & Cantor (1969) model of sequences evolution using Model-testing feature of MEGA5 software (Tamura et al. 2011). A bootstrap consensus tree (Fig. 5) was inferred from 1000 replicates, and corresponding bootstrap values >50% are cited in the tree.

Taxonomy

Tylopilus sultanii S. Sarwar, Khalid & Niazi, **sp. nov.** Figs 2–3

MycoBank MB 802339

> Differs from *Tylopilus pseudoscaber* by its context that does not change color when bruised, its larger basidiospores, and its cracked pileus surface.
>
> Type: Pakistan, Khyber Pakhtunkhwa, Ayubia, 2350 m a.s.l., under *Pinus wallichiana* A.B. Jacks., on ground, 15 August 2006, A.R. Niazi 36 (**Holotype**, LAH0806; GenBank KJ775786).
>
> Etymology: referring to Dr. Sultan Ahmad, a pioneer mycologist in Pakistan.

Pileus 6–9 cm wide, globose, hemispherical to broadly convex, dark brown, surface dry, rough, cracked into rectangular patches, context pinkish yellow to creamy visible between patches, margins straight to deflexed, same color as pileus surface, even margins. Context creamy, no color change upon exposure. Odor and taste not distinctive. Stipe about 5 cm long, 3 cm thick, central, solid, hard, smooth, slightly curved, the center of stipe dark brown to black, at top and base whitish to creamy in color, context like pileus, no color change upon

Figure 2. *Tylopilus sultanii* (holotype, LAH0806).
a, b. Sporocarps, in situ. c,d. Sporocarps. Scale bars = 1.5 cm.

exposure. Pore surface whitish brown to dark brown, adnate and horizontal to sinuate, pores 2–3 per mm, tubes whitish to off-white, 9–17 mm deep, no color change to slightly brown upon bruising. Edibility not known.

Basidiospores oblong–inequilateral, apiculate, smooth, 18–19 × 8–10 µm, (18.4 ± 0.45 × 9.13 ± 0.74; Q_m = 2.07 ± 0.18). Basidia clavate, 2–4-sterigmate, sterigmata long, contents visible, brownish pigments visible, 62–64 × 19–20 µm. Cystidia cylindrical to subclavate, thin walled, hyaline, 32–35 × 9–10 µm. Pileipellis a layer of long cylindrical septate hyphae, 76–80(–97) × 10–12 µm.

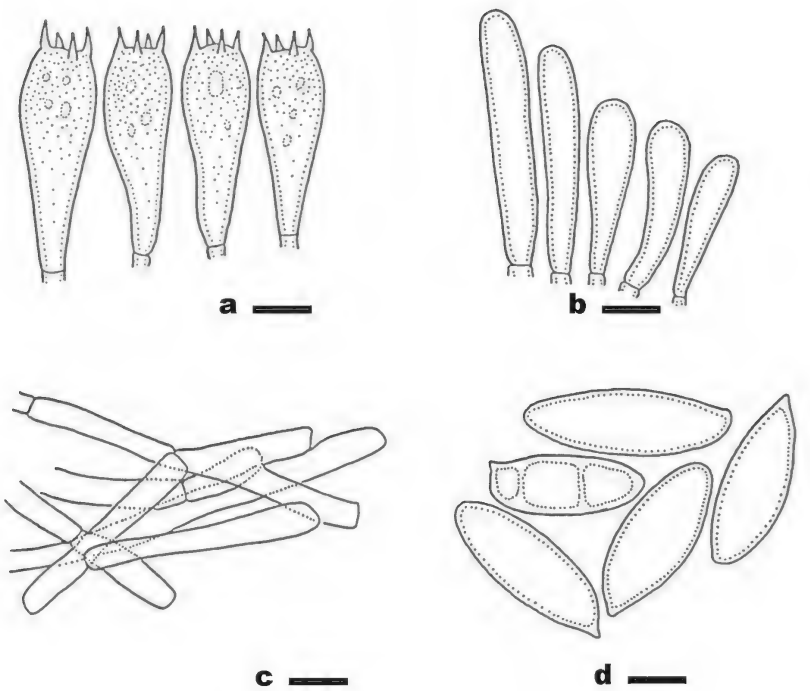

Fig. 3. *Tylopilus sultanii* (holotype, LAH0806).
a. Basidia. b. Cystidia. c. Pileipellis hyphae. d. Basidiospores.
Scale bars: a = 16 µm; b = 9.5 µm; c = 17 µm; d = 6 µm.

Chemical reactions: pileipellis staining reddish brown in KOH; spores yellowish brown to reddish brown in Meltzer's reagent.

Ecology & distribution: Known only from the type locality, fruiting July–September during rainy season.

Notes: *Tylopilus sultanii* is similar to *T. badiceps* (Peck) A.H. Sm. & Thiers, *T. indecisus* (Peck) Murrill, and *T. pseudoscaber.* However, *T. badiceps* differs by its obliquely truncate pileus, stipe with a whitish apex, reticulations near the apex, and spores without an apiculus; *T. indecisus* differs by its pale brown to dull cinnamon pileus surface and reticulated stipe; and *T. pseudoscaber* differs by its context bluing upon bruising and its smaller basidiospores (Thiers 1975, Bessette et al. 2000, Lakhanpal 1996).

Tylopilus pseudoscaber (Singer) A.H. Sm. & Thiers, Mycologia 60: 950 (1968) Fig. 4
≡ *Porphyrellus pseudoscaber* Singer, Farlowia 2: 115 (1945)

Pileus 5–9 cm wide, convex to broadly convex, surface dry, smooth, dull, dark olive brown to dark brown, margins smooth, entire, same color as pileus, deflexed to slightly incurved. Context white to off–white, slowly bluing upon exposure, and then turning brownish. Stipe 6–11 cm long, 1.5–3 cm thick, subclavate to equal, straight to slightly curved, dry, dull, solid, centric, color like pileus surface, whitish near base, sometimes brownish dots present, context off-white, change to brownish when exposed. Pore surface dark reddish brown to blackish brown, staining dark blue at first then turning dark brown, pores 2–3/mm, circular to angular, narrow, 2–3 per mm, tubes 1–2.5 cm deep, shorter towards margins, brownish. Smell pungent, Taste not distinctive. Edibility not known.

Basidiospores ellipsoid to subfusoid, smooth, thick walled, 11–16 × 6–8 µm, (13.6 ± 1.6 × 7.07 ± 0.72; Q_m = 2.05 ± 0.37). Basidia clavate, 3–4 sterigmate, thick walled, 34–41 × 11–13 µm. Cystidia subclavate to fusoid to ventricose, with tapering tips, brownish in KOH, 39–59 × 11–17 µm. Pileipellis cylindrical interwoven hyphae, 52–58 × 5–8 µm; most terminal elements cylindrical with pointed tips in some cases, 43–50 × 7–9 µm.

Chemical reactions: pileipellis staining yellowish red in KOH, Meltzer's reagent; spores ochraceous in Meltzer's reagent, light yellow in lactic acid.

Ecology: In Pakistan, fruiting under coniferous trees, July–September during the rainy season.

Material examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, Khaira gali, 2347 m a.s.l., solitary on ground under *Abies pindrow* Royle, 17 July 2009, Sarwar S.B. 20 (LAH0709: GenBank KJ775785); Nathiagali, 2439 m a.s.l., solitary on ground under *Pinus wallichiana* A.B. Jacks., 19 July 2009, Sarwar S.B. 20A (LAH0709); Sharan (Kaghan valley), 2011 m a.s.l., solitary on ground under *Pinus wallichiana* A.B. Jacks., 7 August 2011, Tayiba A. 68 (LAH0811).

Notes: *Tylopilus pseudoscaber* is similar to *T. porphyrosporus* (Fr.) A.H. Sm. & Thiers, *T. indecisus*, and *T. sordidus* (Frost) A.H. Sm. & Thiers. However, *T. porphyrosporus* differs by its larger size and its context not bluing upon bruising; *T. indecisus* differs by its pale brown to dull cinnamon pileus surface and reticulated stipe; and *T. sordidus* differs by its longitudinal streaks on the stipe (Bessette et al. 2000, Thiers 1975).

Key to *Tylopilus* species recorded from Pakistan

1. Stipe with brownish base, with reticulations ..... *T. felleus*
1. Stipe with whitish base, without reticulations 2
2. Basidiospores 11–16 × 6–8 µm; context bluing upon bruising ..... *T. pseudoscaber*
2. Basidiospores 18–19 × 8–10 µm; context not bluing upon bruising ..... *T. sultanii*

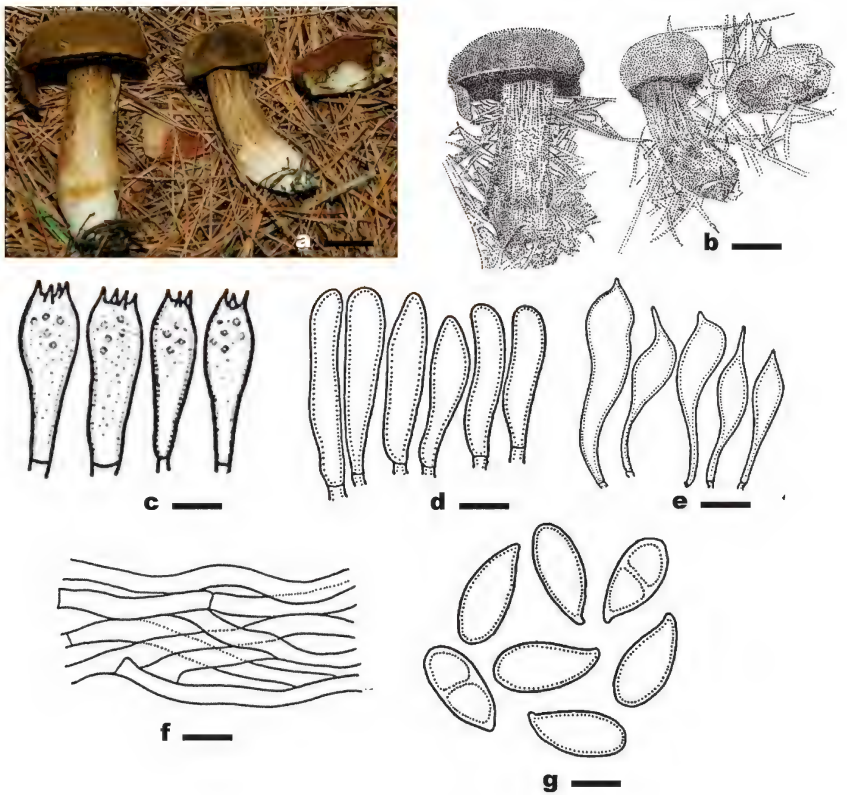

FIGURE 4. *Tylopilus pseudoscaber* (LAH0709).
a, b. Sporocarps. c. Basidia. d. Cystidia. e. Terminal elements of pileipellis hyphae.
f. Pileipellis hyphae. g. Basidiospores.
Scale bars: a, b = 3.5 cm; c = 8 µm; d, e = 13 µm; f = 14 µm; g = 7 µm.

Phylogenetic analysis

The closest BLAST matches for our two ITS sequences were *T. pseudoscaber* (JF899578) and *Porphyrellus pseudoscaber* (EU685112); our *T. pseudoscaber* (KJ775785) sequence showed 99% similarity with both of these, but our *T. sultanii* (KJ775786) sequence showed only <95% similarity.

The maximum likelihood phylogenetic tree (FIG. 5) had 674 genetic characters after alignment and trimming from both 3 and 5 sites of rDNA–ITS. No characters were excluded from the final analysis, all characters were of the 'unord' type, all gaps were treated as "missing" data, and multistate characters were interpreted as uncertain. The phylogram contained three clades; both of our sequences clustered in clade I.

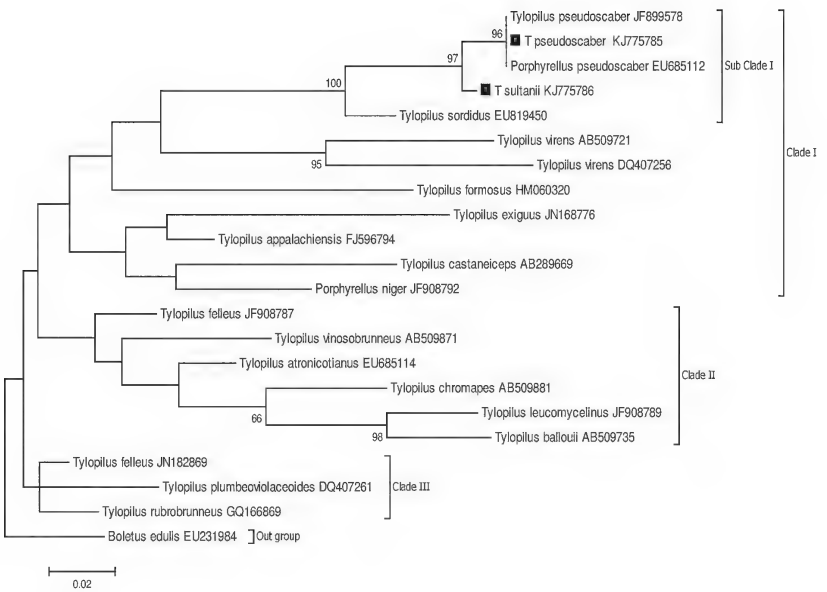

FIGURE 5. Phylogenetic position of *Tylopilus sultanii* and *T. pseudoscaber* from Pakistan relative to other *Tylopilus* spp., with *Boletus edulis* as outgroup. Tree inferred by maximum likelihood analysis based on 5.8S+ITS2 rDNA sequences. Numbers against branches indicate percentage support (>50%) in 1000 bootstrap replications. GenBank accession numbers stand after species names. ▪ = new sequences from Pakistan.

Tylopilus sultanii was shown to be phylogenetically closest to *Porphyrellus pseudoscaber* (EU685112), with which it shares 91.2% genetic characters while being separated by a 1.9% genetic divergence. There was significant genetic divergence of rDNA–ITS between *T. sultanii* and all other rDNA sequences included in the present study. Maximum similarity and shared genetic characters were well below the 97% cutoff value for species delimitation, and genetic divergences were high enough to confirm this species as new.

Our *T. pseudoscaber* sequence shared 99.9% genetic characters with both *T. pseudoscaber* (JF899578) and *Porphyrellus pseudoscaber* (EU685112) with only 0.2% genetic divergence.

Discussion

Both morphological and molecular analysis confirm *T. sultanii* as a new species, forming a highly supported sister clade to *T. pseudoscaber*.

Our sequence analysis confirmed our morphological identification of our Pakistani *T. pseudoscaber* collections, which formed a very highly supported clade with North American sequences of *T. pseudoscaber.* This is a new record for Pakistan.

Acknowledgments

We are thankful to reviewers, Prof. Dr. Robert Laurence Wick (University of Massachusetts, Amherst, USA) and Dr. Najam-ul-Sehar Afshan (University of the Punjab, Lahore, Pakistan). We are also very grateful to the Higher Education Commission of Pakistan for providing financial support for this research work and Jodrell Laboratories (Royal Botanic Gardens Kew) for providing facilities for molecular work.

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Anacraspedodidymum, a new genus from submerged wood in Brazil

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Abstract — *Anacraspedodidymum aquaticum* gen. et sp. nov., found on submerged decaying wood in Riacho do Mel, Bahia, Brazil, is described and illustrated. It is characterized by monophialidic, integrated, terminal conidiogenous cells, with a funnel shaped collarette and solitary, obovoid, globose to elliptical, slightly apiculate at the base, aseptate, hyaline conidia with irregular to subreticulate mucous ornamentation. A new combination, *Anacraspedodidymum hyalosporum,* is proposed to accommodate *Craspedodidymum hyalosporum.*

Key words — microfungi, freshwater, taxonomy

Introduction

During research on conidial fungi associated with submerged decaying plant material in Riacho do Mel, municipality of Alagoinhas, Bahia, Brazil, an interesting fungus was found, distinguished by phialidic conidiogenous cells and conidial features that show some similarities with the genus *Craspedodidymum* Hol.-Jech. but which morphologically differs from all described species. Therefore, it is described as new to science.

Samples of submerged litter were placed in paper and plastic bags. In the laboratory the samples were placed in Petri dish moist chambers and stored in a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol, at 25°C for 30 days (Castañeda-Ruiz 2005). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements were made at a magnification of

×1000. Micrographs were obtained with an Olympus microscope (model BX51) equipped with bright field and Nomarski interference optics. The type specimens are deposited in the Herbarium of Universidade Estadual de Feira de Santana (HUEFS).

Taxonomy

Anacraspedodidymum C.R. Silva, R.F. Castañeda & Gusmão, **gen. nov.**

MycoBank 805402

Differs from *Craspedodidymum* by its hyaline basal apiculate conidia, sometimes with mucilaginous adherences.

Type species: *Anacraspedodidymum aquaticum C.R. Silva et al.*

Etymology: Greek, *Ana-*, meaning upwards, back and again; Latin, *-craspedodidymum* referring to the hyphomycete genus *Craspedodidymum*.

Colonies effuse, hairy, brown to black. Mycelium superficial and immersed. Conidiophores macronematous, mononematous, unbranched, straight to slightly flexuous, septate, smooth or verruculose, brown to pale brown. Conidiogenous cells monophialidic, integrated, terminal, cylindrical, sometimes with percurrent elongations; collarette funnel shaped or infundibuliform, brown. Conidia solitary, aseptate, solitary, ellipsoid, spherical, globose, obpyriform to obovoid, with an inconspicuous basal hilum or slightly papillate, sometimes with mucous adherences or tunicate ornamentation, hyaline.

Anacraspedodidymum aquaticum C.R. Silva, R.F. Castañeda & Gusmão, **sp. nov.** Plate 1

MycoBank 805403

Differs from *Craspedodidymum* spp. by its hyaline conidia with irregular or subreticulate mucilaginous ornamentations.

Type: Brazil, Bahia State, Alagoinhas, Riacho do Mel, 12°10′S 38°24′W, on submerged decaying wood in a stream, 18 Oct. 2011, coll. C.R. Silva (**Holotype**: HUEFS 196431).

Etymology: *Latin, aquaticum,* refers to its growing in water.

Colonies effuse, hairy, brown. Mycelium partly superficial, partly immersed in the substratum. Hyphae septate, smooth, pale brown, 2.5 µm diam. Conidiophores macronematous, mononematous, simple, unbranched, straight to slightly flexuous, 2–7-septate, smooth, brown to pale brown at apex, 64–219 × 3–5 µm. Conidiogenous cells monophialidic, integrated, terminal, cylindrical, sometimes with percurrent elongations, 17–63 × 3–4 µm; collarette funnel shaped, 4–5 × 2–3 µm, pale brown. Conidia solitary, ellipsoid, spherical to obovoid, aseptate, with irregular or subreticulate mucilaginous ornamentation, hyaline, 8–12 × 7–8 µm, mostly slightly papillate at the base.

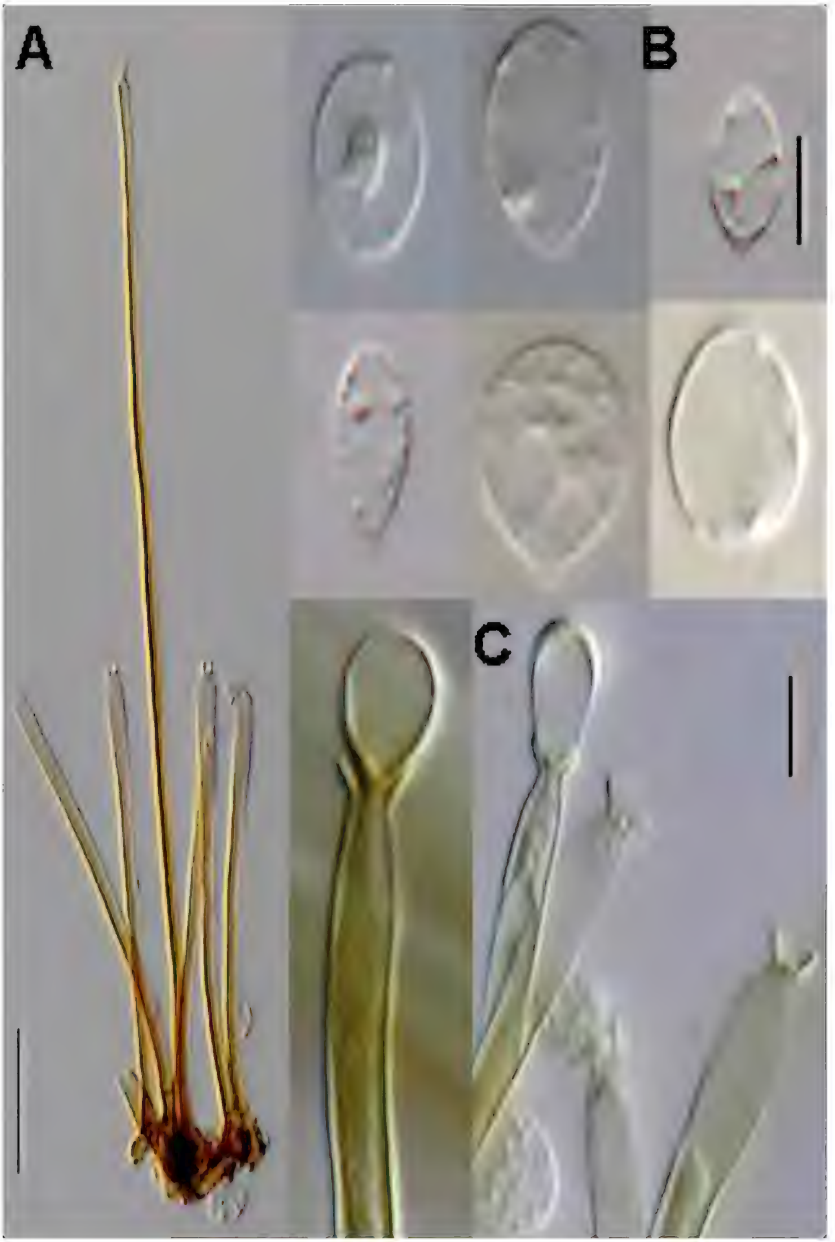

Fig. 1. *Anacraspedodidymum aquaticum* (holotype, HUEFS 196431). A. Conidiophores. B. Conidia. C. Conidiogenous cells and conidia. Scale bars: A = 20 μm; B–C = 5 μm.

Additional specimen examined: **BRAZIL**, **Bahia**, Riacho do Mel, 12°10′S 38°24′W, on submerged wood, 18 Oct. 2011, coll. C.R. Silva (HUEFS 196430).

Notes: Holubová-Jechová (1972) and Seifert et al. (2011) described *Craspedodidymum* (type = *C. elatum* Hol.-Jech.) with dichotomously branched or single conidiophores and phialidic terminal conidiogenous cells with a funnel-shaped collarette; the conidia are solitary, 0–4-septate, brown to dark brown. The *Craspedodidymum* generic concept was enlarged to accommodate *C. hyalosporum*, a species with 1-septate, hyaline conidia (Bhat & Kendrick, 1993) and which differs from all other described *Craspedodidymum* species (Yanna et al. 2000, Ma et al. 2011). We propose a new combination in *Anacraspedodidymum*.

Anacraspedodidymum hyalosporum *(*Bhat & W.B. Kendr.*)* R.F. Castañeda, C.R. Silva & Gusmão, **comb. nov.**

MycoBank 805404

≡ *Craspedodidymum hyalosporum* Bhat & W.B. Kendr., Mycotaxon 49: 35 (1993).

Bahusutrabeeja Subram. & Bhat, *Cylindrotrichum* Bonord., *Kylindria* DiCosmo et al., and *Monilochaetes* Halst. are also superficially similar to *Anacraspedodidymum*, but *Bahusutrabeeja* has aseptate hyaline conidia with several filiform extracellular appendages. *Cylindrotrichum* has polyphialidic conidiogenous cells, while monophialidic conidiogenous cells are present in *Kylindria*, but blastic sympodial extension frequently occurs within the collarette in both genera (Castañeda-Ruiz & Kendrick 1990, Kirk et al. 2008, Seifert et al. 2011). *Monilochaetes* has monophialidic conidiogenous cells, with an inconspicuous or evident collarette and cylindrical to oblong conidia that accumulate in mucous masses or are (rarely) pseudocatenulate (Reblová et al. 2011, Seifert et al. 2011)

Acknowledgments

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Two new rust species on *Fabaceae* from Brazil

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Abstract— Two new species of rust fungi (*Basidiomycota*, *Pucciniales*) on *Fabaceae* are proposed: *Atelocauda sakuraguiae* on *Ormosia* sp. and *Ravenelia costae* on *Pseudopiptadenia leptostachya*. Both were collected at the Parque Nacional do Itatiaia, Brazil. *Atelocauda sakuraguiae* can be distinguished from all other *Atelocauda* species on *Fabaceae* by its non-lobed aeciospores, septate aecial paraphyses, less elongate teliospores, and septate teliospore pedicels. *Ravenelia costae* can be distinguished from all other *Ravenelia* species by its smooth teliospores, probasidia deriving from germ pores, pendent cysts, echinulate urediniospores, and absence of paraphyses.

Key Words— Atlantic forest, biodiversity, phytopathology, plant disease, *Uredinales*

Introduction

Through a project supported by the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro entitled "Uredobiota (fungi) do Parque Nacional do Itatiaia," rust fungi specimens were collected in the States of Minas Gerais and Rio de Janeiro, Brazil. This field and laboratory work revealed two species with distinctive characteristics differing from other rust species on *Fabaceae* that are described here as new.

Materials & methods

The specimens were collected during 2006 and 2007 in the Parque Nacional do Itatiaia in the States of Minas Gerais and Rio de Janeiro, Brazil. Collections were slowly dried and accessioned into the Herbarium of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RB). Sori were observed using a Leica S6E stereomicroscope. Free hand sections and scrapings were placed in lactophenol or chloral hydrate and then examined using a Zeiss Axioskop 40 compound microscope. For scanning electron microscopy (SEM) observation, material was mounted on a double-sided adhesive tape, fixed in osmium vapor for 17 h, gold-palladium coated with an Emitech K550X coater,

and observed with a Zeiss EV 550X SEM. Illustrations were created from images of these slides.

Taxonomy

Atelocauda sakuraguiae Salazar-Yepes & A.A. Carvalho, **sp. nov.** FIGS 1–8

MYCOBANK MB564578

Differs from all other *Atelocauda* species on *Fabaceae* by its non-lobed aeciospores, septate aecial paraphyses, less elongate teliospores, and septate teliospore pedicels.

TYPE: on *Ormosia* sp. (*Fabaceae*): Brazil, Minas Gerais, Parque Nacional do Itatiaia, Vale de Santa Clara Trail, 22°19′17.4″S 44°37′17.8″W, 1615 m asl, 11 Oct 2007, M. Salazar Yepes & A.A. Carvalho Jr. 638-07 (**Holotype**, RB480639).

ETYMOLOGY: Dedicated to Cassia Mônica Sakuragui, a distinguished botanist and authority on *Araceae*.

Spermogonia few, amphigenous, mostly on adaxial leaf surface, Group VI (type 7). Aecia *Uredo*-type, few amphigenous, mostly on adaxial leaf surface, subcuticular, in groups, erumpent, cinnamon-brown. Paraphyses abundant, peripheral, incurved, flexuous, septate at base, 81–90 × 9–12 µm; wall smooth, colorless to pale yellow. Aeciospores obovoid to clavate, 27–30 × 21–27 µm; wall echinulate, 1.5–2 µm thick at sides, 2–3 µm thick at base, chestnut gold; germ pores obscure, one near the hilum. Telia on abaxial leaf surface, scattered, or in groups, minute, subcuticular in origin, becoming ruptured with conspicuous ruptured cuticle, pulverulent, chocolate-brown, with abundant peripheral paraphyses. Paraphyses cylindrical, flexuous, incurved, dichotomically and trichotomically branched, septate at base, 75–105 × 12–15 µm; wall smooth, mostly dorsally thickened up to 6 µm, and colorless to pale yellow. Teliospores obovoid to oblong-globoid, 24–33 × 24–30 µm; distal tubercles, 5 µm wide; wall uniformly 2–3 µm thick, cinnamon brown, tuberculate; germ pores one near the hilum; pedicel fragile, septate near the spore, ≤75 µm long, colorless.

ECOLOGY — The rust infects *Ormosia* plants at altitudes above 1600 m above sea level (asl) in National Park of the Itatiaia, whose forest is designated as dense rainforest High Montana (Veloso et al. 1991).

COMMENTS — Arthur & Cummins (1933) erected *Atelocauda* (with *A. incrustans* Arthur & Cummins as its type) differing from *Uromyces* by its type 7 spermogonia and from *Dicheirinia* and *Diabole* by its one-celled teliospores formed on pedicels. Thirumalachar & Kern (1955) reduced the genus to synonymy under *Pileolaria* and recombined its type as *P. incrustans*. Although Cummins (1959, 1978) accepted the placement in *Pileolaria*, Cummins & Hiratsuka (1983) resurrected *Atelocauda*, to which they transferred three *Uromyces* species on *Acacia* from Hawaii, Australia, and neighboring regions. Walker (2001), who re-circumscribed *Atelocauda*, transferred five species assigned to *Atelocauda* by Cummins & Hiratsuka (1983), Ono (1984), and

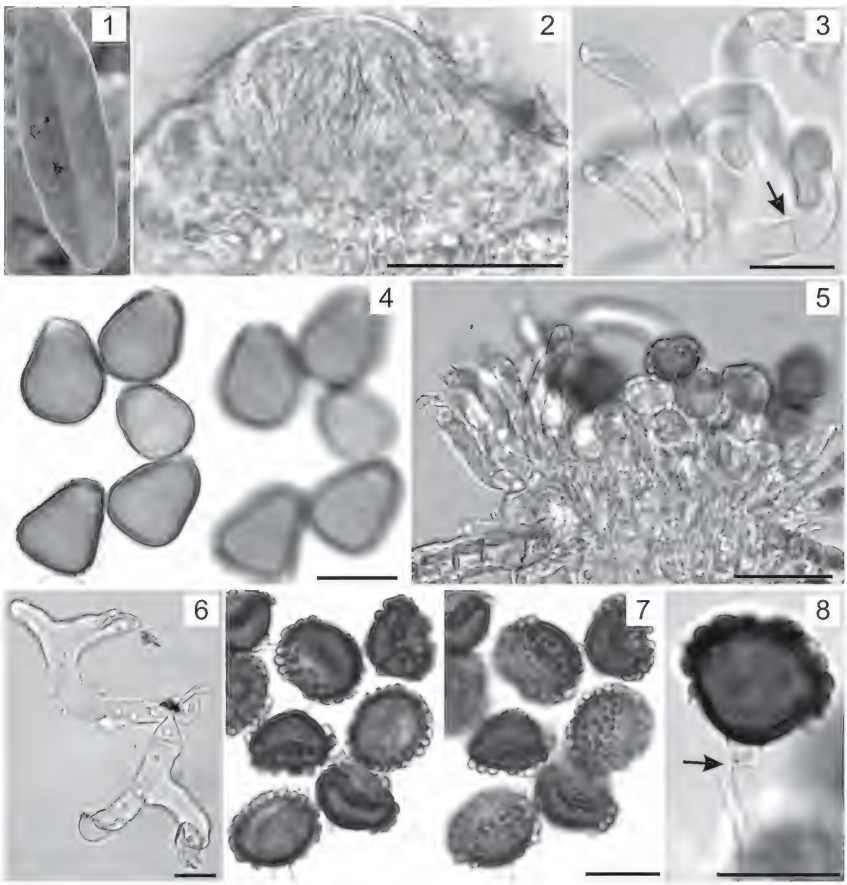

FIGURE 1–8. *Atelocauda sakuraguiae* (holotype, RB480639). 1. Aecial (*Uredo*-type) symptoms on adaxial leaf surface. 2. Section of spermogonium (type 7). 3. Flexuous paraphyses showing a septum (arrowed). 4. Aeciospores; left, median focus; right, surface focus. 5. Section of telial sorus, showing teliospores and paraphyses. 6. Dichotomous telial paraphyses. 7. Teliospores; left, median focus; right, surface focus. 8. Teliospores; pedicel septate near the spore (arrowed). Bars: 2, 5 = 50 µm; 3, 4, 7, 8 = 20 µm; 6 = 10 µm.

Gardner (1991) to a new genus *Racospermyces* and added a new species, *A. shivasii* J. Walker on *Ormosia* from Australia. Zhuang & Wei (2009) recombined *Uromyces ormosiae* L. Guo & Y.C. Wang on *Ormosia* spp. as *Atelocauda ormosiae*, based on its subcuticular spermogonia type 7.

Atelocauda shivasii, *A. incrustans*, and *A. ormosiae* differ from *A. sakuraguiae* by their lobed anamorph spores, their non-septate teliospore pedicels, and their more elongated teliospores (Arthur & Cummins 1933, Walker 2001, Zhuang & Wei 2009).

Walker (2001) also discovered spermogonia and *Uredo*-type aecia with lobed spores on the type specimen of *A. incrustans* and pointed out the morphological similarities between *Atelocauda shivasii* on *Ormosia* and *Dicheirinia ormosiae* (Arthur) Cummins, whose urediniospores are similar in shape with a basal germ pore associated with a smooth patch. Similarly *Atelocauda sakuraguiae* has *Uredo*-type aecia and aeciospores with a basal germ pore in a smooth area (Fig. 4). From these morphological similarities, it is assumed that *Atelocauda* is more closely related to *Raveneliaceae* than to *Pileolariaceae* (sensu Cummins & Hiratsuka 2003). Like Walker (2001), we consider *Atelocauda* to be closely related to *Ravenelia*, *Dicheirinia*, and other genera in *Raveneliaceae*.

Ravenelia costae Salazar-Yepes & A.A. Carvalho, **sp. nov.** Figs 9–14

Mycobank MB564579

Differs from all other *Ravenelia* species by its smooth teliospores, probasidial cells germinating by germ pores, pendent cysts, echinulate urediniospores, and absence of paraphyses.

Type: on *Pseudopiptadenia leptostachya* (Benth.) Rauschert (*Fabaceae*): Brazil, Rio de Janeiro, Itatiaia, Parque Nacional do Itatiaia, around the bridge over the Rio Campo Belo, 22°27′41.1″S 44°37′9.5″W, 1109 m asl, 6 Aug 2007, M. Salazar Yepes & A.A. Carvalho Jr. 469-07 (**Holotype**, RB532381).

Etymology: Dedicated to Denise Pinheiro da Costa, a distinguished botanist and authority on *Metzgeriaceae*, *Hepaticae*.

Spermogonia amphigenous, in groups on rounded necrotic lesions, 1–2 mm diam. Aecia dark cinnamon brown, on abaxial leaf surface, scattered, or in groups, peridiate, cupulate, white to pale yellow. Peridial cells irregular in shape, firmly united, 21–48 µm diam.; outer wall finely verrucose and inner wall verrucose-striate, colorless. Aeciospores obovoid to oblong-ellipsoid, polyhedric, 30–42 × 24–27 µm; wall verrucose, 1–2 µm thick, pale chestnut brown to colorless. Uredinia on abaxial leaf surface, scattered, or in small groups, minute, subepidermal in origin, ruptured epidermis conspicuous, pulverulent, cinnamon-brown; paraphyses absent. Urediniospores obovoid, pyriform, ellipsoid, 30–45 × 18–24 µm; wall echinulate, 1–2 µm thick at sides, 1–3 µm thick at apex, pale cinnamon-brown; germ pores obscure. Telia on abaxial leaf surface, scattered, or in small groups, subepidermal in origin, ruptured epidermis conspicuous, pulverulent, dark cinnamon brown; paraphyses absent. Teliospore heads 45–90 µm diam., 4–7 probasidial cells across, central cells 18–21 µm diam., variable in size and shape; wall smooth, 2–3 µm thick, cinnamon-brown; cysts colorless, globoid, pendent, 21–30 µm diam.; germ pores evident, 1 in each cell, 3–6 µm diam.; pedicel multi-hyphal, mostly persistent, ≤75 µm, hyaline.

Additional specimens examined: on *Pseudopiptadenia leptostachya*: **Brazil**, **Rio de Janeiro**, Itatiaia, Parque Nacional do Itatiaia, on the Véu de Noiva-Itaporani

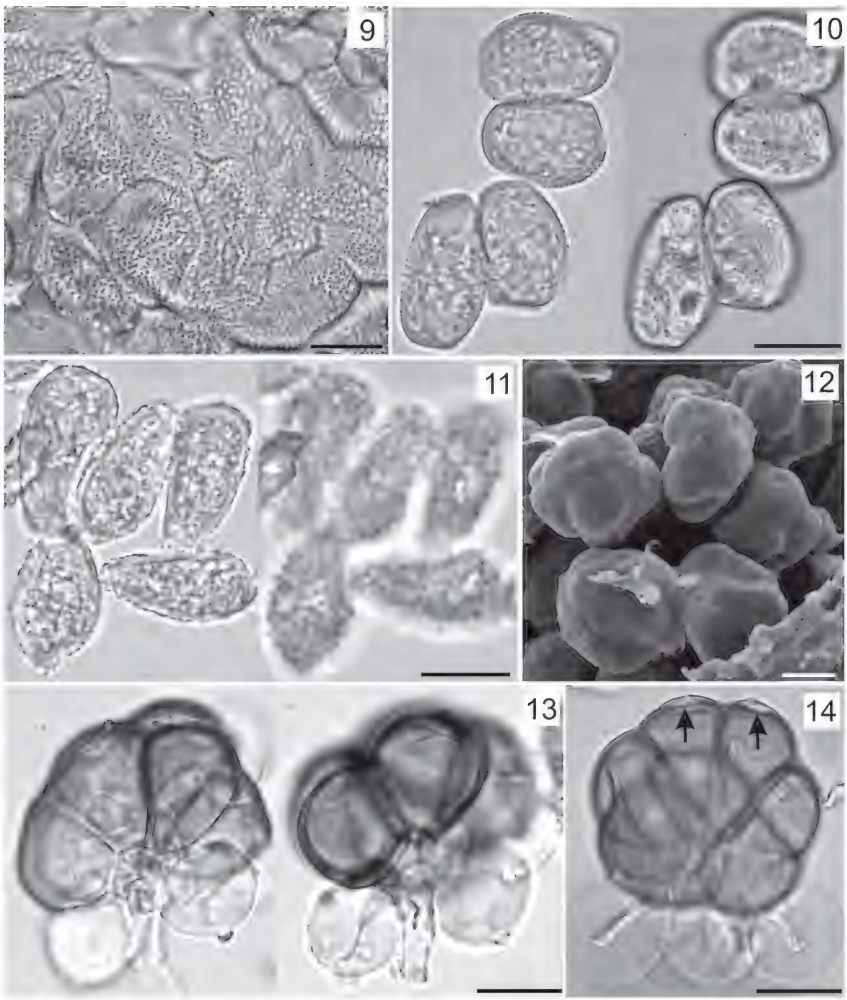

FIGURE 9–14. *Ravenelia costae* (holotype, RB532381). 9. Peridial cells. 10. Aeciospores; left, median focus; right, surface focus. 11. Urediniospores. Left, median focus; right surface focus. 12. Teliospores; upper view (SEM). 13. Teliospores; left, median focus; right, surface focus. 14. Teliospores; apical pores (arrowed). Bars = 20 µm.

Waterfalls Trail, 1180 m asl, 17 Aug 2006, M. Salazar Yepes, A.A. Carvalho Jr. & I. França 227-06 (RB480597); 23 Apr 2007, M. Salazar Yepes, A.A. Carvalho Jr. & F. Santoro 211-07 (RB480593); Rio Campo Belo-Prateleiras Trail, 22°25′44.8″S 44°37′37.6″W, 1400 m asl, 5 Dec 2006, M. Salazar Yepes & A.A. Carvalho Jr. 448-06 (RB480621); 22°26′7.3″S 44°37′32.5″W, 1265 m asl, 27 Apr 2007, M. Salazar Yepes, A.A. Carvalho Jr. & F. Santoro 355-07 (RB480612); around the Abrigo 10 (Lamedo), 22°25′34.4″S 44°37′54.1″W, 1500 m asl, 5 Dec 2007, M. Salazar Yepes & A.A. Carvalho Jr. 725-07 (RB480644).

Ecology — The rust-infected *P. leptostachya* plants are found at altitudes under 1500 m in National Park of the Itatiaia, whose forest is classified as dense rainforest Montana (Veloso et al. 1991).

Comments — *Pseudopiptadenia*, the host of our species, belongs to the Piptadenia group in the *Mimosoideae* subfamily of legumes. *Mimosoideae* hosts have at least 73 species of *Ravenelia*. From these, only seven have smooth teliospores and uredinia that (if present) lack paraphyses. *Ravenelia versatilis* (Peck) Dietel and *R. chacoensis* J.C. Lindq. differ from *R. costae* by their larger teliospores; *R. bahiensis* Henn., *R. hieronymi* Speg., and *R. entadae* Lagerh. & Dietel differ by their teliospores having germ slits, and *R. bahiensis* also differs by being microcyclic; *R. striatispora* Cummins & J.W. Baxter differs by its urediniospores having striate ornamentation; and *R. prosopidicola* J.C. Lindq. differs by its smaller aeciospores with 6–7 equatorial germ pores (Hennen et al. 2005, Resende 2007, and Hernandez et al. 2012).

After comparison with the most comprehensive recent literature, including Hennen et al. (2005), Resende (2007), and Hernandez et al. (2012), we determined that *R. costae* is unique among other *Ravenelia* by the combination of smooth teliospores, probasidial cells germinating by germ pores, pendent cysts, echinulate urediniospores, and uredinia without paraphyses.

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The first record of *Hebeloma vinosophyllum* (*Strophariaceae*) in Southeast Asia

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Abstract – Basidiomata of a *Hebeloma* sp. were collected from two urea-plots in a pine (*Pinus kesiya*) forest, Da Lat City, Lam Dong Province, Vietnam. Based on morphology, molecular phylogeny, and mating compatibility, the specimens were identified as *Hebeloma vinosophyllum*. This is the first record of *H. vinosophyllum* in Southeast Asia and of *H. vinosophyllum* occurring in *P. kesiya* forests, one of two dominant pines in Southeast Asia.

Key words – *Porphyrospora*, cortina, cheilocystidia, ammonia fungi

Introduction

Hebeloma vinosophyllum was originally described by Hongo (1965) from Japanese specimens. This species was reported to resemble *H. sarcophyllum* (Peck) Sacc. in having a light brown to purplish red spore print but to differ in the shape of the hymenial cystidia (Hongo 1965, Singer 1986, Vesterholt 2005). In 1975, Sagara reported *H. vinosophyllum* as a member of the "ammonia fungi

... a chemo-ecological group of fungi that develop reproductive structures exclusively or relatively luxuriantly on soil after a sudden addition of ammonia, of other nitrogenous materials that react as bases by themselves or on decomposition, or of alkalis" (Sagara 1975). Basidiomata of *H. vinosophyllum* were recorded not only from the forest floor sites augmented with urea but also from soils naturally disturbed by decomposition of dead animal bodies (Sagara 1976, 1992, Takayama & Sagara 1981, Fukiharu et al. 2000a,b).

Hebeloma vinosophyllum has been recorded from different vegetation in various geographical regions of Japan. The fungus has been collected from *Castanopsis*, *Quercus*, and *Pinus* forests (Table 1) in middle and western Honshu (Hongo 1965, Sagara 1975, Suzuki 1987, Yamanaka 1995, Fukiharu et al. 1995, Fukiharu et al. 2000a,b, Kasuya 2002, Imamura & Yumoto 2004), and Shikoku and Kyushu (Sagara 1975). However, this fungus has not been recorded for urea applications in *Pinus* and *Quercus* forests in Hokkaido (Sagara 1975) or eastern Honshu (Sagara 1975, Fukiharu & Horigome 1996), or in *Castanopsis* and *Quercus* forests on Ryukyu Island (Fukiharu & Hongo 1995).

Outside Japan, *H. vinosophyllum* has been recorded from a fagaceous-coniferous forest (*Quercus*, *Castanopsis*, *Castanea*, *Cunninghamia*, *Pinus*, etc.) near SanMing City, Fujian Province, China (Hongo et al. 1996; no voucher specimen deposited – Dr. Noriko Kinjo, pers. comm.) and possibly in South Korea (Lee 2011; species name listed without details). These collection records suggest that *H. vinosophyllum* has an East Asian distribution (Fukiharu & Horigome 1996).

During a survey of ammonia fungi in southern Vietnam, specimens belonging to *Hebeloma* sect. *Porphyrospora* Konrad & Maubl. ex Vesterh. (Vesterholt 2005) were collected. We identified these Vietnamese specimens based on morphology, molecular phylogeny, and compatibility between the strains from Vietnam and stock cultures of *H. vinosophyllum* from Japan.

Materials & methods

Collection

Urea-treated plots were established in a *Pinus kesiya* Royle ex Gordon forest (more than 30 years old), ca. 1500 m altitude, at Xuan Tho, Da Lat City, Lam Dong Province, Vietnam. Commercial granulated urea fertilizer was applied to 2 plots (1 × 1 m) on the forest floor in the amount of 600 g/m^2. About two months after urea application, basidiomata of *Hebeloma* sp. were observed, collected, and subcultured onto agar medium. Cultures were maintained on MY agar medium [malt extract 10 g/L (Difco, Detroit, USA), yeast extract 2 g/L (Difco, Detroit, USA) and agar 15 g/L (Nacalai Tesque, Japan)] at 20°C in darkness. Basidiomata were dried at 60°C for 24 h and deposited in the herbarium of the Natural History Museum and Institute, Chiba, Japan (CBM). Cultures were deposited in Faculty of Biology, University of Science, Ho Chi Minh City, Vietnam (HCMUS).

Other specimens and cultures of *H. vinosophyllum* in Japan, *H. aminophilum* in Australia, and *H. porphyrosporum* in Italy were deposited in the Natural History Museum and Institute, Chiba, Japan (CBM); Forest Microbiology Division, Forestry and Forest Products Research Institute, Ibaraki, Japan (CHU); and Biological Resource Center, National Institute of Technology and Evaluation, Japan (NBRC).

Observation

Morphological observations were recorded from both dry and fresh field specimens. Color notation follows Kornerup & Wanscher (1981).

The microscopic characteristics were observed using differential interference contrast (DIC) on Labophot-2 (Nikon, Tokyo, Japan) or Olympus B51 (Tokyo, Japan) microscopes after hand-sectioning and mounting in 10% ammonium hydroxide aqueous solution. The basidiospores were also observed by a scanning electron microscope (SEM) (Hitachi S-800; Tokyo, Japan) at 15.0 kV after rehydrating in 25% aqueous ammonia, fixing in 2.5% osmic acid, and coating with platinum-palladium in an ion sputter-coater (E-1030; Hitachi, Tokyo, Japan). Abbreviations used: Q = mean length/width ratio measured from "n" number of spores; m = mean spore length and width.

Phylogenetic analysis

Total nuclear DNA (nDNA) was extracted from disintegrated tissue of dried mycelia and basidiomata using 200 µl 0.5 mm glass beads (Yasui Kikai, Tokyo, Japan) and 500 µl TES buffer [50 mM Tris-HCl, pH 7.5, 20 mM EDTA, 1% sodium dodecyl sulfate (SDS)] by vigorous shaking (FastPrep System; mp-Biomedicals, Solon, OH, USA) at 6.5 m/s for 45 s. Soluble fractions were recovered by centrifugation. DNA was purified using TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)–saturated phenol/chloroform/isoamyl alcohol (25:24:1, Nippon Gene, Tokyo, Japan) extraction followed by an isopropyl alcohol precipitation. After desiccation of the DNA pellet, DNA was dissolved in a 30 µl TE buffer. For some samples, genomic DNA was further purified using NucleoSpin Extract II (Macherey-Nagel, Duren, Germany), following the manufacturer's recommendations.

The primer pairs ITS1 and ITS4 or ITS5 and ITS4 (White et al. 1990) were used to amplify the ITS regions of ribosomal DNA (rDNA). The primers LR0R and LR5 (Vilgalys and Hester 1990) were used to amplify 28S rDNA (large subunit, LSU). Polymerase chains reactions (PCR) were carried out using KOD FX (Toyobo, Tokyo, Japan) following the manufacturer's instructions. PCR products were purified using NucleoSpin Extract II, and DNA fragments were directly sequenced using the BigDye Terminator ver3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the protocol provided. Reactions were cleaned up using the Centri Sep (Princeton Separations, Adelphia, NJ, USA), before analyzing by capillary electrophoresis on a 3130x DNA Analyzer (Applied Biosystems). Sequences were assembled and edited using ATSQ software (Genetyx, Tokyo, Japan) and deposited in GenBank/EMBL/DDJB (Table 1).

Two data sets (LSU, ITS) were established that included sequences from Vietnamese specimens, several sequences of Japanese *H. vinosophyllum,* and sequences downloaded from GenBank (Table 2). The data sets were aligned using Clustal X ver. 1.81 (Jeannmougin et al. 1998), and the resulting alignments were manually refined. For phylogenetic analyses, each data set (LSU = 892 bp, ITS = 625 bp) was analyzed using

Table 1. Collection details of *Hebeloma* specimens and cultures in the phylogenetic analysis.

Taxon	Voucher no./Isolate no.	Origin	GenBank acc. no.	
			ITS	LSU
H. vinosophyllum	CBM:FB32636*/CBM-BC69	Japan	AB1742172	AB1742341
	CBM:FB12306*/CBM-BC307	Japan	AB1742173	AB1742342
	CBM:FB12325*/CBM-BC314	Japan	AB1742174	AB1742343
	CBM:FB12381*/CBM-BC315	Japan	AB1742175	AB1742344
	CBM:FB14335*/CBM-BC337	Japan	AB1742176	AB1742345
	CBM:FB14285*/CBM-BC366	Japan	AB1742177	AB1742346
	CBM:FB14216*/CBM-BC376	Japan	AB1742178	AB1742347
	CBM:FB14520*/CBM-BC384	Japan	AB1742179	AB1742348
	CBM:FB14502*/CBM-BC407	Japan	AB1742180	AB1742349
	CBM:FB15552*/CBM-BC437-3	Japan	AB1742181	AB1742350
	CBM:FB15553*/CBM-BC438-2	Japan	AB1742182	AB1742351
	CBM:FB15556*/CBM-BC440-2	Japan	AB1742183	AB1742352
	CBM:FB24700/CBM-BC533**	Japan	AB1742184	AB1742353
	—/NBRC107913	Japan	AB1742185	AB1742354
	—/CHU4001*	Japan	AB1742186	AB1742355
	CBM:FB39191*/HCMUS-C1	VietNam	AB1742187	AB1742356
	CBM:FB39192*/—	VietNam	AB1742188	AB1742357
	CBM:FB39267*/HCMUS-C2	VietNam	AB1742189	AB1742358
H. aminophilum	—/CHU5001*	Australia	AB1742190	AB1742359
	—/CHU5002***	Australia	AB1742191	AB1742360
	CBM:FB35472*/—	Australia	AB1742192	AB1742361
H. porphyrosporum	CBM:FB24804/—	Italy	AB1742193	AB1742362

Basidiomata appeared * in urea plot, ** around decaying unidentified animal, or *** around decaying snake.

Tree-Puzzle 5.2 (Schmidt et al. 2002). The maximum likelihood (ML) tree (Felsenstein 1981) of each data set was inferred based on quartet puzzling algorithm (Strimmer & Haeseler 1996) with the options of 1000 puzzling steps, model of substitution HKY (Hasegawa et al. 1985).

Table 2. Published sequences in the phylogenetic analysis

Taxon	GenBank acc. no.	
	ITS	LSU
Hebeloma fastibile	AF325643	AY033139
H. sarcophyllum	AF124715	——
H. vinosophyllum (NBRC32945)	[03294501]*	——
H. vinosophyllum (Boyle et al. 2006)	AY320398**	——
Alnicola lactariolens	AY818352	AY818353
Anamika angustilamellata	AY575919	AY575919
An. indica	AF407163	AF407164

* Retrieved from DNA resource of Biological Resource Center, National Institute of Technology and Evaluation, Japan.

** Used only for alignment with sequence from Vietnamese *H. vinosophyllum*.

Mating test

The dikaryotic stock culture of Vietnamese *Hebeloma* sp. (HCMUS-C2) was cultured on Ohta's medium (Ohta 1990) with 15 g agar, at 20°C, 12 h/12 h light/dark cycle. Light was irradiated at ca. 140 μmol $m^{-2}s^{-1}$. Basidiomata appeared in vitro after ca. 3 weeks, when a small piece of sterile filter paper was placed under the hymenophores for collecting basidiospores. Monokaryotic strains were obtained by germinating these in vitro spores.

Mating tests between monokaryotic tester strains of Vietnamese *Hebeloma* sp. and dikaryotic stock cultures of Japanese *H. vinosophyllum* (isolates CBM-BC69 and CHU4001) were compared. Mating types were determined by pair-mating with each other. The mating tests were conducted by placing a pair of mycelial discs (a monokaryotic tester strain vs. a dikaryotic culture) on opposite sides of 90 mm MY agar plates, incubated at 25°C in darkness, and replicated 3 times. After two weeks, mycelium from the monokaryotic colony was removed from the edge farthest from the dikaryotic colony and examined microscopically; a compatible crossing was indicated by the presence of clamp connections.

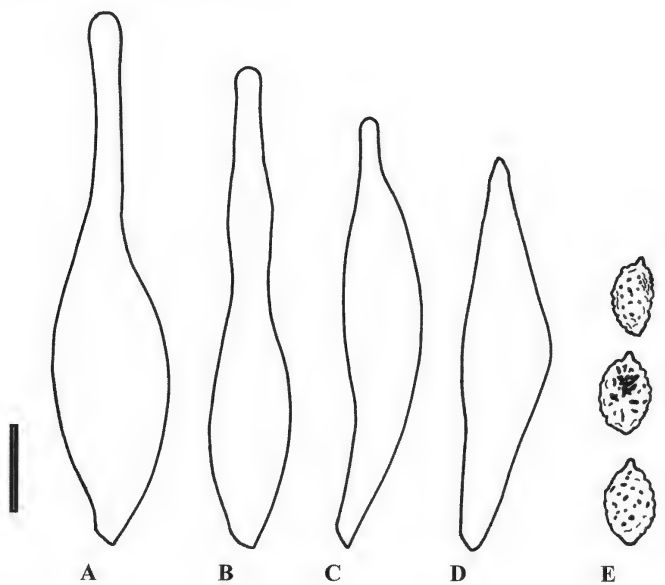

Plate 1. *Hebeloma vinosophyllum*. A–D: Hymenial cystidia. E: Basidiospores. Scale bar = 10 μm.

Taxonomy

Hebeloma vinosophyllum Hongo, J. Jap. Bot. 40: 314. 1965 Plates 1–2

[Description based on Vietnamese specimens] Pileus 20–70 mm broad, at first hemispherical, then becoming plano-convex to plane; cream to light

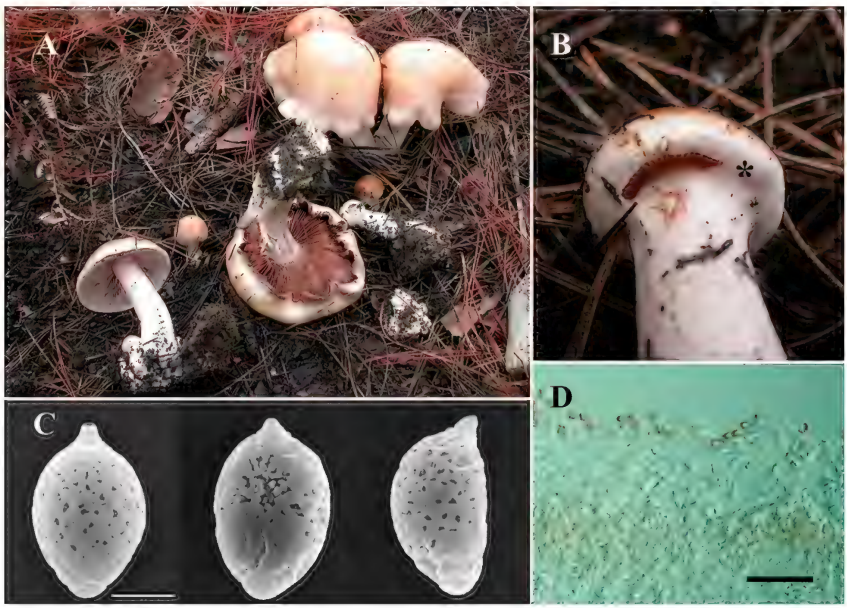

Plate 2. *Hebeloma vinosophyllum* from the urea plot in Viet Nam. A: Basidiomata in nature. B: Cortina (partial veil; *) of a young basidioma. C: Scanning electron micrographs of basidiospores. D: Ixotrichodermium structure of pileipellis. Scale bars: C = 3 µm; D = 30 µm.

vinaceous (7–9A2–3), usually darker at the top; surface glabrescent, smooth, viscid to glutinous in wet condition; margin at first inrolled and entire, then plane or eroded-undulating, sometimes still incurved at maturity. Universal veil absent, partial veil fibrillose, white, dry, at first cortinoid (following Vesterholt 2005), leaving a few fibrils on the stipe, disappearing with age. Lamellae crowded, 2–4 mm width, 5–30 mm length, adnexed; pinkish brown (8–9C3–5) to light vinaceous (8–9A3–5) and darker at maturity. Stipe up to 105 x 15 mm, thickened downward; surface somewhat smooth to longitudinal striate, floccose-pruinose at the apex; fleshy-fibrous, stuffed at first, then hollow; cream (7A2–3) to light clay (5D5–6). Context pale white (7–8A2–3); smell musty; taste weakly bitter. Spore print vinaceous to brownish red (9–10C3–4).

Basidiospores m = 9.7 ± 0.6 × 6.1 ± 0.4 µm (8.4–11.3 × 5.3–7.2 µm, n = 50, Q = 1.59), amygdaliform to citriform. Basidia 30–40 × 8–9 µm (without sterigmata), clavate, four-spored; sterigmata 3–5 µm long. Pleurocystidia 48–60 × 8–12 µm, numerous, ventricose-rostrate to lageniform, narrowly utriform or mucronate. Cheilocystidia similar to pleurocystidia but less numerous. Pileipellis an ixotrichodermium, composed of gelatinized cylindrical cells 50–60 µm in length; hypodermium cellular composed of globose cells 7–9 µm in width.

Habitat – In Vietnam, scattered, gregarious, or subcespitose on urea-plots in *Pinus kesiya* forest, ca. 1500 m, 2 months after urea application. Elsewhere in warm temperate East Asia scattered, gregarious, or subcespitose on soil under *Castanopsis*, *Quercus*, and *Pinus*, usually 6-12 months after urea application or following decomposition of dead animal bodies.

Distribution (Plate 3): Japan (middle to western Honshu, Shikoku, and Kyushu); China (SanMing, Fujian Province); Vietnam (DaLat City, LamDong Province); possibly South Korea (confirmation needed).

Specimens examined: **VIETNAM. Lam Dong**: Da Lat City, Xuan Truong - Xuan Tho (UTM, 49P, 023053, 1319015), 24 Jun 2009, B.N Truong (CBM FB-39191; culture HCMUS-C1); 25 Jun 2009, B.N Truong (CBM FB-39192); 14 Jun 2009, B.N Truong (CBM FB-39267; culture HCMUS-C2). **JAPAN. Shiga**: Otsu City, Ishizune, 17 Jul 1961, T Hongo (TNS-F-39101, holotype)

Plate 3. Biogeographic distribution of *Hebeloma vinosophyllum* in Japan (Honshu: Saitama, Tochigi, Tokyo, Chiba, Shiga, Shizuoka, Kyoto, Tottori; Shikoku: Kochi; Kyushu: Oita, Miyazaki), South Korea, China (Fujian Province), and Vietnam (Da Lat). Δ = no deposition of voucher specimen, O = collection site of voucher specimen.

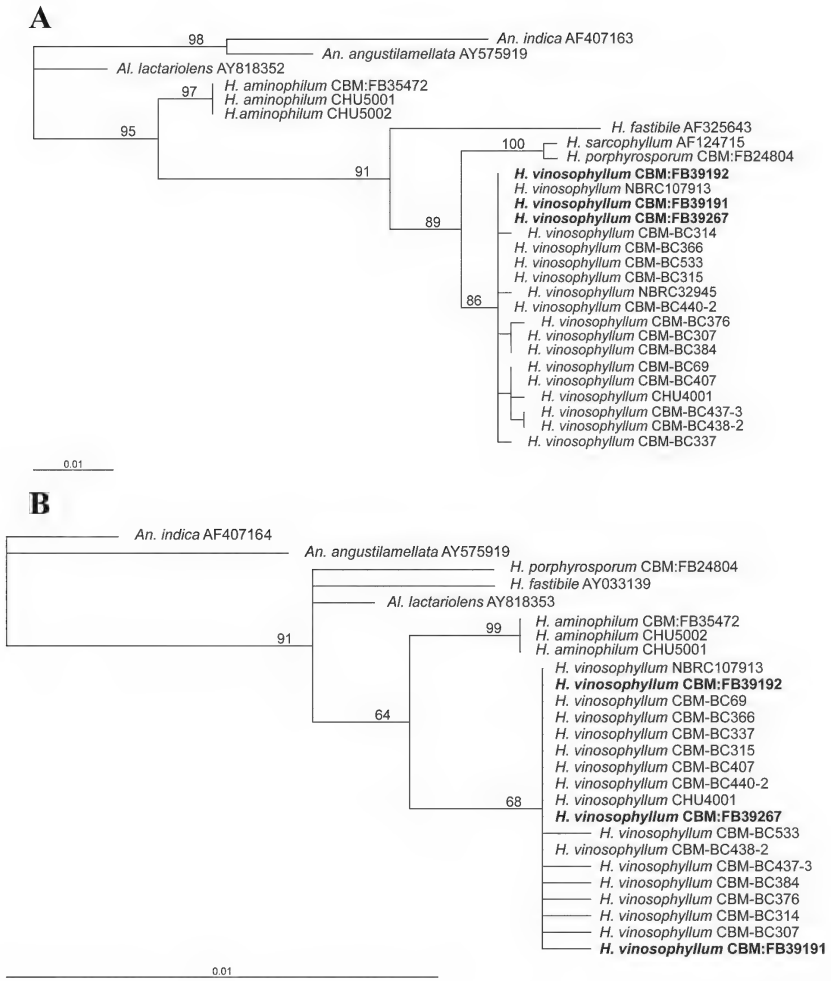

Plate 4. The maximum likelihood phylogenetic tree based on ITS data set (A) and LSU data set (B) of *Hebeloma vinosophyllum* and allied species. The numbers on each branch represent bootstrap percentage support values. The scale bar shows the ratio substitutions/site. Trees are rooted by outgroup *Anamika indica* and *An. angustilamellata*. Vietnamese specimens are in bold. Abbreviation: *Al.* = *Alnicola*.

Phylogenetic analysis

Dikaryotic cultures and dried specimens of Vietnamese *H. vinosophyllum* have identical ITS and LSU sequences. Sequence alignments of dikaryotic *H. vinosophyllum* cultures from Japan and dried *H. vinosophyllum* specimens

from Vietnam showed 99.55% (887/891 bp) similarity of the LSU and 98.52% (600/609 bp) similarity of the ITS regions. The phylogenetic tree (Plate 4) grouped the dikaryotic cultures and the Vietnamese specimens in the same clade as those from Japan (supporting values = 68% in LSU and 86% in ITS).

We could not analyze the holotype specimen (TNS-F-39101) of *H. vinosophyllum* molecularly due to its long storage with para-formaldehyde. The lower ITS similarity of 95.4% (587/615 bp) for GenBank AY320398 (Boyle et al. 2006, labeled as *H. vinosophyllum*) suggests that that material is misidentified.

Mating test

The di-mon mating tests between monokaryotic strains from Vietnam and dikaryotic cultures of Japanese *H. vinosophyllum* showed clamp connections (Table 3), indicating that all Vietnamese monokaryotic strains were compatible with Japanese *H. vinosophyllum* cultures.

Table 3. Dikaryon-monokaryon mating tests between *Hebeloma vinosophyllum* (Japan) and *Hebeloma* sp. (Vietnam)*

		Monokaryotic strains of Vietnamese *Hebeloma* sp. HCMUS-C2							
		A_1B_1		A_2B_2		A_1B_2		A_2B_1	
		C2-03	C2-36	C2-02	C2-37	C2-09	C2-38	C2-01	C2-35
Dikaryotic stock cultures of Japanese *H. vinosophyllum*	CBM-BC69	+	+	+	+	+	+	+	+
	CHU4001	+	+	+	+	+	+	+	+

*+ = clamp connections formed

Discussion

Except for forming more abundant cheilocystidia, all Vietnamese specimens were similar to the holotype specimen of *H. vinosophyllum* (TNS-F-39101) based on morphological analysis. The di-mon mating tests indicate that Vietnamese *Hebeloma* sp. belongs to the same biological species as *H. vinosophyllum*. The phylogenetic analyses (Plate 4) also indicate that all Vietnamese and Japanese specimens belong to the same species.

This is the first record of *H. vinosophyllum* in Southeast Asia (Plate 3) and of *H. vinosophyllum* associating with *P. kesiya,* one of two dominant pines in Southeast Asia (Zonneveld et al. 2009).

As a putative ectomycorrhizal fungus (Fukiharu 1991, Sagara 1995, Imamura & Yumoto 2008), *H. vinosophyllum* could habituate from hosts of

Northeast Asian fagaceous and/or pinaceous species to those of Southeast Asian *Pinus* species, or vice versa. Further studies of applications of urea to forests up to the northern limit of *P. kesiya* in central Asia (Zonneveld et al. 2009) and to fagaceous (possibly *Quercus* and *Castanopsis*) and pinaceous forests in Ryukyu islands, Taiwan, southern China, and Southeast Asia will help resolve the geographic range, host tree species, and potentially the origin of *H. vinosophyllum*.

Despite the presence of a cobweb-like partial veil (cortina), *H. vinosophyllum* could belong to *Porphyrospora* (Vesterholt 2005) based on its brownish red spore deposit and amygdaliform-citriform spores.

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Xylaria hongkongensis sp. nov. from an urban tree

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Abstract — A new species of *Xylaria* from Hong Kong, China, is described based on its teleomorphic characters and is distinguished by its conical stromata and small spores. The fungus was found on the trunk of a living *Bauhinia variegata* in an urban park, and the infected tree exhibited overall declining symptoms including branch dieback and leaf discoloration.

Key words — taxonomy, wood decay fungi, *Xylariales*

Introduction

Trees in cities constitute the most prominent elements of urban nature due to their seasonal changes, size, shape, and color (Tyrväinen et al. 2005). However, they are beset by many aerial and subterranean stresses, making them susceptible to fungi (Bradshaw et al. 1995, Luley 2005, Sinclair & Lyon 2005). Loss of mechanical strength caused by fungal damage in urban areas can be linked to hazardous situations such as tree wind throws or limb failures, resulting in significant property damage and injuries. Therefore, timely assessment and accurate identification of wood decay fungi are important for assessing structural stability and predicting the probability of tree failure or decline.

During an assessment of trees on an urban slope, a species of *Xylaria* was found on a declining *Bauhinia variegata*. The tree exhibited dieback symptoms with leaf discoloration and browning. Numerous stromata emerged on one dead trunk and along bark cracks of the declining trunk. Morphological features showed that this fungus represents an undescribed species of *Xylaria*, which is described and illustrated here.

Materials & methods

Fungal samples were collected on a slope of Waterfall Bay Park, Waterfall Bay Road, Wah Fu, Southern District, Hong Kong, China. Microscopic observations were made in water, and Melzer's reagent was used to test the amyloidity of apical ring. Measurements were taken at ×400 magnification on samples of 30 ascospore, 20 asci, and 10 perithecia. A herbarium specimen was deposited in the Institute of Microbiology, Chinese Academy of Science, Guangzhou, China (GDGM), and a living culture placed in Centraalbureau voor Schimmelcultures, The Netherlands (CBS).

Taxonomy

Xylaria hongkongensis A.M.C. Tang, R.Y.C. Lam & M.W.K. Leung, **sp. nov.** Fig. 1

MycoBank MB 807096

Differs from *Xylaria pallide-ostiolata* by its cylindric-clavate stromata with conical apices and shorter and lighter-colored ascospores.

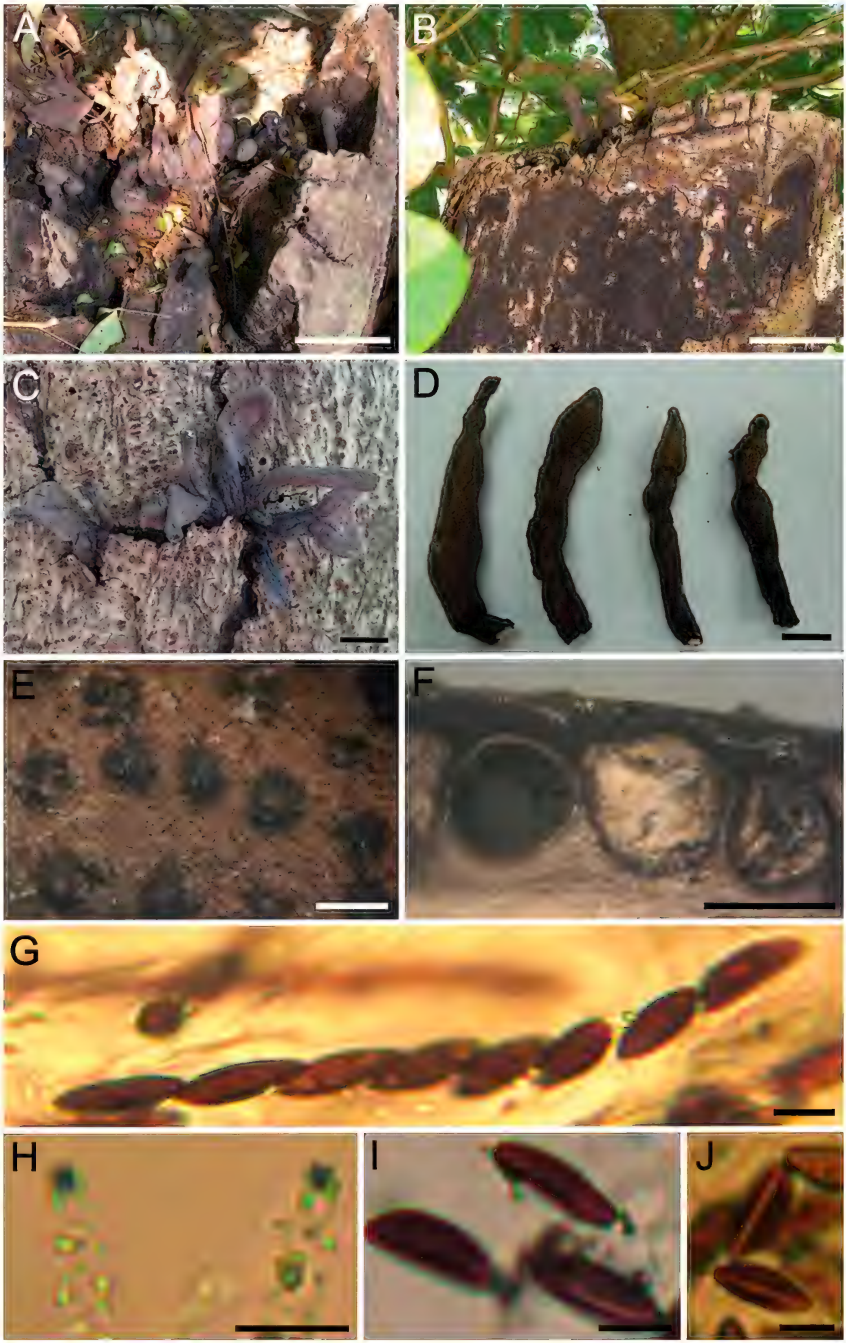
Type: China, Guangdong Province, Hong Kong, Southern District, Wah Fu, Waterfall Bay Road, Waterfall Bay Park, on trunk of *Bauhinia variegata* L. *(Caesalpiniaceae)*, 14.I.2011, A.M.C. Tang (**Holotype**, GDGM40058; ex-type culture, CBS 136698; GenBank KF926669).

Etymology — Referring to the type locality region.

Stromata upright, solitary or caespitose, unbranched, nearly sessile to slightly stipitate, cylindric-clavate, sterile apices conical to subglobose, 38–67 mm total length, 8–12 mm diameter; surface dark brown, roughened with wrinkles. Perithecia with slightly exposed outlines, subglobose, 410–840 µm high, 410–690 µm diam. Ostioles slightly papillate or inconspicuous. Asci 8-spored, cylindrical, long-stipitate, 120–147.5 µm total length, 3.75–7.5 µm width, the spore-bearing part 67.5–95 µm long, with apical apparatus bluing in Melzer's reagent, tubular-shaped, 3–4.5 µm high × 2–3 µm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, 12.5–15 × 5–7.5 µm, smooth, usually with a straight germ-slit nearly spore length on the concave side.

Comments — This new *Xylaria* possesses a combination of morphological characters that do not match any previously described species. Based on its stromatal appearance, *X. hongkongensis* should be categorized in the *X. polymorpha* complex. Its ascospores resemble those of *X. pallide-ostiolata* Henn. (12–18 × 6–7 µm; San Martín & Rogers 1989: 356) and *Xylaria* sp. SM303 (12.5–14.5 × 5–5.5 µm; San Martín & Rogers 1989: 364–365), but *X. pallide-ostiolata* differs by its broadly clavate stromata with rounded apices

Fig. 1. *Xylaria hongkongensis* (holotype, GDGM40058). a–b: Stromata on dead trunk. c: Stromata emerged on cracks of trunk. d: Herbarium specimen GDGM40058. e: Stromatal surface with ostioles. f: Section through stroma, showing perithecial layer. g: Ascus in water. h: Apical apparatus of immature asci stained in Melzer's reagent. i: Ascospores observed in water. j: Ascospores observed in water showing germ slit morphology. Scale bars: a–b = 5 cm; c–d = 1 cm; e–f = 500 µm; g–j = 10 µm.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and its longer black spores, while *Xylaria* sp. SM303 differs by a germ-slit that is much less than spore length and its longer asci (148–166 µm). *Xylaria scruposa* (Fr.) Fr., which has similarly sized stromata (15–60 × 2–6 mm), differs by its narrow stroma with a highly roughened surface and longer ascospores (18–22 × 6–7 µm; San Martín & Rogers 1989: 363).

It is noteworthy that *X. hongkongensis* was found in association with trunk and basal rot in a common urban tree during our tree risk assessment. This association reminds us that some *Xylaria* species could manifest themselves as pathogens on weak hosts, especially in susceptible urban plantations. Since *Xylaria* species are ubiquitous endophytes of most vascular plants (Petrini et al. 1995), under favourable conditions *Xylaria* species might change their passive ecological strategy into an active mode (Promputtha et al. 2007). *Xylaria* species have long been recognized as causing root rot on alfalfa, apple, crabapple, eastern redbud (*Cercis canadensis*), hedge maple (*Acer campestre*), pear, and thornless honeylocust (*Gleditsia triacanthos* var. *inermis*) (Rogers 1984, Proffer 1998). Thus, it is important to stay vigilant on trees showing signs of stress.

Acknowledgments

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A new species of *Sphaceloma* on *Helicia* from China

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Abstract—*Sphaceloma heliciae* sp. nov. on *Helicia formosana* is described and illustrated. The type specimen was collected from Xishuangbanna, Yunnan Province, China.

Key words—coelomycetes, identification, taxonomy

Introduction

The genus *Sphaceloma*, proposed by de Bary in 1874, contains 163 named species (Index Fungorum 2013), about 52 of which are currently accepted (Kirk et al. 2008). Many species have *Elsinoe* teleomorphic states. In China, 24 *Sphaceloma* spp. have been reported (Tai 1979; Wang et al. 1999; Guo 2001, 2005). Of the five *Sphaceloma* spp. that have been recorded on proteaceous hosts (*Elsinoe banksiae* Pascoe & Crous, *E. leucospermi* L. Swart & Crous, *E. proteae* Crous & L. Swart, *Sphaceloma banksiicola* Pascoe & Crous, and *S. protearum* L. Swart & Crous), none has been reported on *Helicia* (Swart et al. 2001; Pascoe & Crous 2007). We describe a new species found on leaves of *Helicia* from China, and distinguish it from the other five species on *Proteaceae*. The holotype specimen is conserved in Herbarium Mycologica of Yunnan Agricultural University, Kunming, China (MHYAU).

Sphaceloma heliciae H.Y. Yang & Z.Y. Zhang, **sp. nov.** Fig. 1

MycoBank804587

Differs from the *Sphaceloma* states of *Elsinoe banksiae* and *E. leucospermi* by its shorter, narrower conidiophores and its narrower, fusiform to ovoid conidia.

Type: China, Yunnan, Xishuangbanna, in living leaves of *Helicia formosana* Hemsl. (*Proteaceae*), Dec. 2009, Li-Xin (**Holotype**, MHYAU 13083).

Etymology: referring to the host genus.

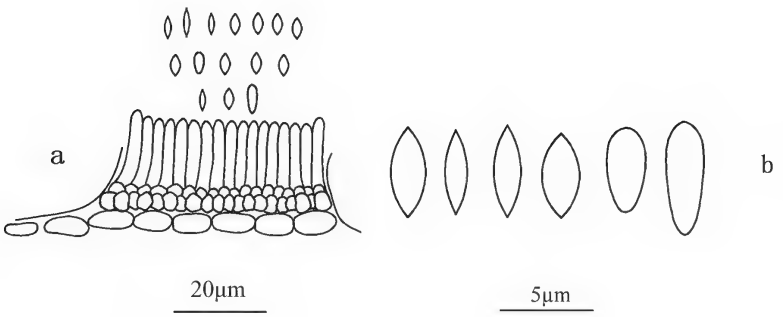

Fig. 1. *Sphaceloma heliciae* (holotype, MHYAU 13083): A. acervulus; B. conidia.

Scabs subrotund, amphigenous, ochroleucous, prominent, 0.8 × 1 mm. Acervuli subcuticular, scattered or aggregated, 31.0 × 51.7 μm, composed of cornuted cells, hyaline to brown. Conidiophores compactly arranged, continuous, hyaline, 13.1–23.9 × 1.7–3.5 μm. Conidia fusiform, ovoid or occasionally cylindrical, continuous, hyaline, 4.6–6.2 × 1.3–2.1 μm.

Discussion

The hosts and morphology of *Sphaceloma heliciae* and the five other species on proteaceous hosts are presented in Table 1. In addition to their association with different host genera, the five other species differ morphologically from *S. heliciae* by their broader ellipsoidal conidia and by either their broader conidiophores (*Elsinoe banksiae*, *E. leucospermi*, *S. banksiicola*, and *S. protearum*) or their lack of extended conidiophores (*E. proteae*).

Table 1. Host associations and morphology of *Sphaceloma*/*Elsinoe* species on proteaceous hosts.

Species	Host	Conidiophores (μm)	Conidia (μm)
E. banksiae	*Banksia serrata*	20–30 × 3–5 subcylindrical	4–6 × 2–3 ellipsoid
E. leucospermi	*Leucospermum cordifolium*	20–30 × 3–6 subcylindrical	5–7 × 2.5–3 ellipsoid
E. proteae	*Protea cynaroides*	reduced to conidiogenous cells	hjnnb
S. protearum	*Protea eximia*	12–20 × 5–6 subcylindrical	5–6 × 2–2. 5 ellipsoid
S. banksiicola	*Banksia prionotes*	8–20 × 3–5 subcylindrical	8–9 × 2.5–4 ellipsoid
S. heliciae	*Helicia formosana*	13–23 × 0.7–3.5 subcylindrical	4.6–6.2 × 1.3–2.1 fusiform to ovoid

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Additions to the Chilean phalloid mycota

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Abstract — Two species in the *Phallales* (*Agaricomycetes*), *Laternea pusilla* and *Lysurus cruciatus*, are recorded as new to Chile. We also present a discussion and a key to the phalloid species known to occur in the country.

Key words — *Clathrus*, gasteromycetes, *Ileodictyon*, *Phallus*, stinkhorns

Introduction

Phalloid fungi, commonly known as stinkhorns, may produce basidiomata with very distinct morphologies, but all are characterized by an ovoid structure during the immature stage. Most species produce an expanded basidiome when mature, but others (e.g., in *Protubera* Möller, *Claustula* K.M. Curtis, *Gelopellis* Zeller, maintain the glebal tissue enclosed by the peridium in a sequestrate structure. The gleba is usually mucilaginous and fetid, which facilitates the spores' dispersion by insects.

Phallales E. Fisch. is traditionally classified in two families, mainly based on basidiome development and morphology: *Clathraceae* Chevall. and *Phallaceae* Corda (Fischer 1898-99, Pilát 1958, Kirk et al. 2008). Recent sequence analyses support these two families as monophyletic clades, but four additional families are accepted: *Claustulaceae* G. Cunn., *Lysuraceae* Corda, *Protophallaceae* Zeller, and *Trappeaceae* P.M. Kirk (Hosaka et al. 2006, Kirk et al. 2008).

Phalloids are widespread, but most species are found in the tropics, with South East Asia being the center of diversity (Kreisel 1996, Cannon & Kirk 2007). However, despite their ample diversity and distribution, in Chile they

have been poorly studied. The first report is from the nineteenth century, when Montagne (1853–54: 497–499) recorded the occurrence of species of *Clathrus* P. Micheli ex. L., *Ileodictyon* Tul. & C. Tul., and *Laternea* Turpin. Additional species were reported by foreign naturalists (e.g., Cunningham 1868, 1871; Johow 1896; Spegazzini 1921; Zeller 1939; Santesson 1943; Singer 1969). Reports from Chilean researchers beginning in the twentieth century (Gunkel 1939, Lazo 1983, 2001; Lazo et al. 1977, Garrido 1981, 1986; Parra & Escudero 1994) have increased the number of phalloid species registered in Chile. The aim of this study is to provide descriptions of species previously unrecorded from Chile and a key to facilitate identification of all phalloid species recorded in this country.

Materials & methods

Specimens were treated according to Rossman et al. (1998) and photographed (in and ex situ) with a Nikon Coolpix P8 (Nikon, Japan). Vocabulary for morphological features follows Calonge (1998), and colors were coded following Kornerup & Wanscher (1978). Dried collections were rehydrated in 90% alcohol before examination. Anatomical characters were observed under a phase contrast microscope (Axiostar plus, Carl Zeiss, Germany) on glass slides mounts in water, 3% (w/v) KOH with phloxine, and cotton blue lactophenol. Vouchers were deposited at the herbaria of Museo Nacional de Historia Natural, Santiago, Chile (SGO), and Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (ICN). Herbaria acronyms follow Thiers (2013).

Taxonomy

Laternea pusilla Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 343, 1868. Fig. 1

Immature basidiomata epigeous, ovoid to subglobose, ≤3.0 cm in diam.; exoperidium yellowish brown (5E4, 5F4), membranous, fragile, endoperidium white (5A1) to yellowish white (4A2); one or several rhizomorphs attached at the base. Mature basidiomata epigeous, up to 10 cm high; volva with the same characteristics of the immature basidioma. Receptacle long-elliptic in profile, formed by three, occasionally four columns that are free at the base and united above. Columns up to 8 cm high, 0.4–1.4 cm in diam., pastel red (8A5), reddish gray (8B2), reddish white (8A2) to orange white (6A2), fading towards the base, pale orange (5A3) when dry, with a central, longitudinal furrow; with elongated, delicate crests ≤1.5 cm high, projecting from the columns' external margins, especially near the apex; trapezoid in transversal section, multitubular, tubes 8–10, usually not interconnected, with the widest tubes and openings on the adaxial face. Glebifer situated beneath the arms' junction, orange red (8A8); gleba confined to the glebifer, mucilaginous, olive brown (5E7), fetid.

Basidiospores cylindrical attenuate at one side, 3.75–4.5 × 1.5–2 µm, chlorohyaline, smooth, thin-walled. Volva's exoperidium formed by

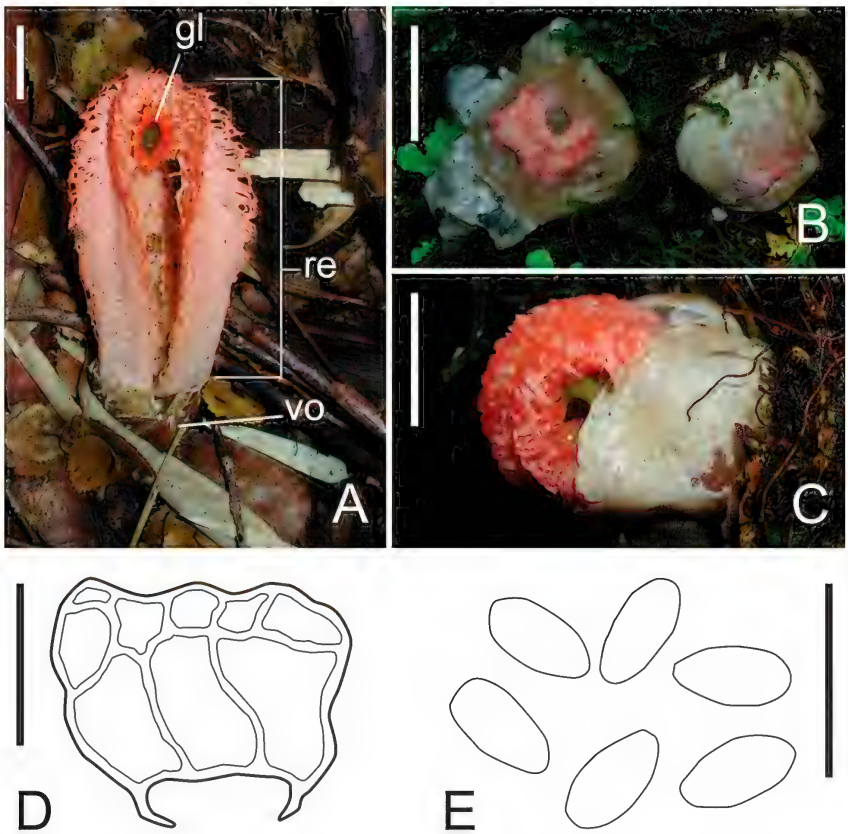

Fig. 1. *Laternea pusilla*: A. Basidiome (gl = glebifer; re = receptacle; vo = volva) (SGO 163170). B, C. Basidiomata expanding from the immature forms (SGO 163171). D. Receptacle column in transverse section. E. Basidiospores (ICN 192157). Scale bars: A–C = 2 cm; D = 2 cm; E = 5 µm.

pseudoparenchymatous hyphae, 14–39 µm in diam., with brownish content, thick-walled, walls yellowish; endoperidium formed by hyaline hyphae, simple septate, 3–7 µm wide, thin-walled. Columns formed by pseudoparenchymatous hyphae, 9–60 µm in diam., hyaline, thin to thick-walled, and walls ≤2.0 µm wide, yellowish to brownish; crests also pseudoparenchymatous.

Habit — growing on mossy barks on shady places. In southern Chile, it is commonly found within *Nothofagus* forests. It is possible that also occurs within *Nothofagus* forests from Argentina.

Specimens examined — **CHILE. Región XI**, Comuna Puerto Aysén, near Lago Yulton, 27.VI.2008, P. Sandoval-Leiva (SGO 163171); near Lago Los Palos, 14.XII.2009, P. Sandoval-Leiva (SGO 163170); **Región X**, Comuna Queilén, Isla Tranqui, 8.II.2009,

P. Sandoval-Leiva (SGO 163169); Chaitén, Parque Pumalín, Sendero de Alerces, 22.XI.2012, L. Trierveiler-Pereira et al. LTP 310 (ICN 192157). **Región XIV**, Comuna Máfil, Fundo Llao-Llao, 12.IX.2011, P. Sandoval-Leiva & J. Marquez (SGO 163168).

Remarks — The most striking feature of *L. pusilla* is the presence of crests projecting from the columns' external margins, usually found on the upper part of the receptacle. When basidiomata are not fresh, crests may appear shrunken. The receptacle morphology varies in many aspects: size, column structure (arched or more or less straight), number of columns (2–4), and color (ranging from bright red to pale pinkish). Chilean specimens are larger than those reported from the neotropics, reaching up to 10 cm high. It is also important to note that sometimes the columns are pitted. *Laternea pusilla* is a neotropical species reported from Central and South America (Dring 1980, Sáenz & Nassar 1982, Meijer 2006).

Lloyd (1909) included seven species in *Laternea*, but Dring (1980) recognized only two (*L. pusilla* and *L. triscapa*). In this study, we recognize a third species, *L. dringii* A. López et al. (López et al. 1981).

Laternea triscapa Turpin was earlier reported from Chile (Montagne 1853–54, Berkeley 1868, Philippi 1869), but we could not confirm the presence of this species in the country. Santesson (1943) suggested that the Chilean specimens identified as *L. triscapa* were misdeterminations of *Colonnaria columnata* (Bosc) E. Fisch [≡ *Clathrus columnatus*]. Dring (1980) examined Santesson's specimens and preferred to keep the name *L. triscapa*; however, he noticed that the specimens had some differences from the tropical ones. It is probable that these *Laternea* specimens from southern Chile reported by Santesson are conspecific with *L. pusilla*. Gamundí & Horak (2002) reported *L. triscapa* for the Patagonian forests in Argentina. The photograph presented by these authors depicts a pinkish receptacle with small crests (which appear shrunken) at the columns' margins. It is possible that this photograph also represents *L. pusilla*.

Lysurus cruciatus (Lepr. & Mont.) Henn., Beibl. Hedwigia 41: 172. 1902. Fig. 2

Immature basidiome subhypogeous, globose to obovoid, soft, up to 4 cm in diam.; with numerous mycelial cords attached at the base; exoperidium grayish yellow (1B3), membranous, endoperidium translucent, gelatinous and rather fluid. Mature basidiome epigeous, 6.5 cm high. Pseudostipe cylindrical, without longitudinal ribs, 6.5 cm × 1.8 cm, yellowish white (1A2), internally hollow, externally rough and consisting of one to three layers of intercommunicating tubes, surmounted by 5–8 arms; arms conical, hollow, up to 3 cm long, sometimes of different lengths in the same basidiome, initially united or not at the tips, but usually free when mature, externally brownish orange (6C6), orange white (6A2) at the center; external surface with a whitish, longitudinal, central, smooth groove; internal surface slightly concave and

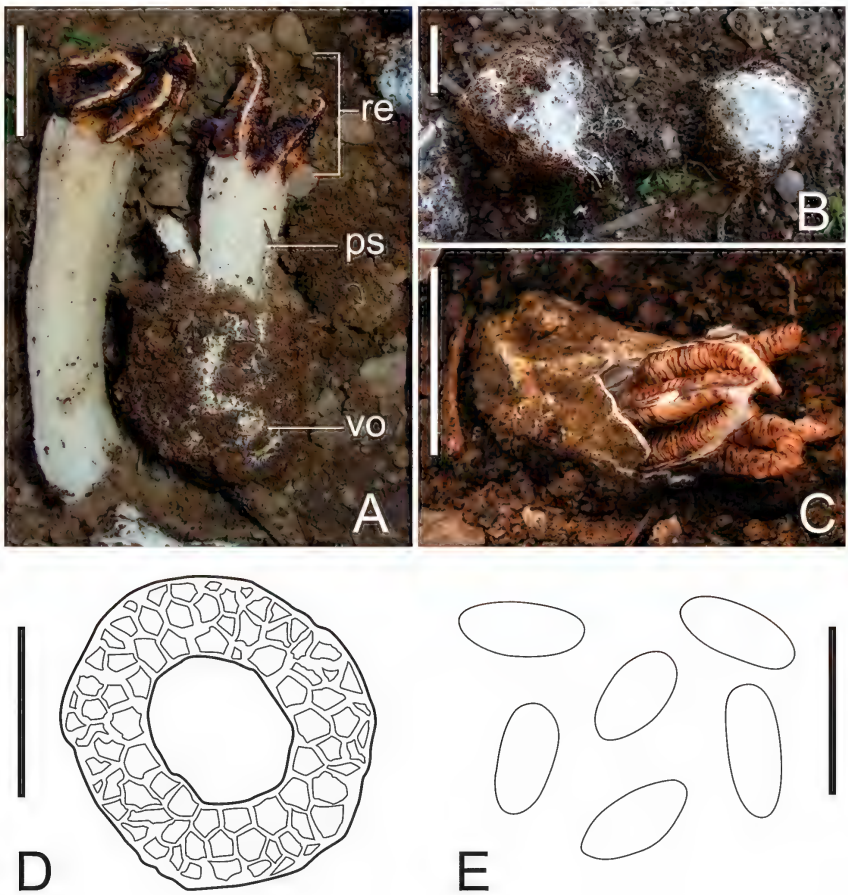

Fig. 2. *Lysurus cruciatus* (SGO 159558): A. Basidiome (re = receptacle; ps = pseudostipe; vo = volva). B. Immature forms. C. Basidiome expanding from the immature form. D. Pseudostipe in transverse section (note the hollow center). E. Basidiospores. Scale bars: A–C = 2 cm; D = 1 cm; E = 5 µm.

covered by the glebal mass. Gleba mucilaginous, yellowish brown (5F5), fetid.

Basidiospores cylindrical to ellipsoid, (3.3–)3.8–4.0(–4.5) × (1.4–)1.7–2.2 µm, chlorohyaline, smooth, thin-walled. Pseudostipe formed by pseudoparenchymatous hyphae, 29–52 µm in diam., hyaline, thick-walled, cell walls up to 5 µm.

Specimen examined — **CHILE. Región Metropolitana**, Melipilla, Cholqui, on soil under *Olea europaea* L., 28.VII.2010, Alarcón, Henríquez & P. Sandoval-Leiva (SGO 159558).

Remarks — The Chilean collection of *L. cruciatus* matches descriptions given by Domínguez (1995), Calonge (1998), and Dring (1980). *Lysurus cruciatus* is a saprobic species that grows in enriched cultivated and uncultivated soils with humus or plant debris in shady and humid places. It has also been recorded in glasshouses (Kreisel 2001). It is a typical alien species, originally described in French Guiana and actually with a wide distribution with records in Australia, Africa, North and South America, Europe, and western Asia (Dring 1980). Kreisel (2001) points out that *L. cruciatus* is unstable in temperate to Mediterranean climates, where it does not expand and disappears after one or several years.

Lysurus Fr. is a widespread genus with five species (Kirk et al. 2008). Prior to this study, there were two records of the genus in Chile: '*Lysurus*? sp.', reported by Spegazzini (1921) based on an immature basidioma; and *Lysurus periphragmoides* (Klotzsch) Dring in *Pinus radiata* plantations (Garrido 1986, as *Simblum sphaerocephalum* Schltdl.).

Discussion

Seven phalloid species are now reported from Chile. Most species have expanded basidiomata, with the exception of *Gelopellis macrospora* Zeller, a hypogeous, truffle-like species.

Phallus impudicus L. is the only species of the genus reported from the country, in the Valdivia province, observed abundantly during several years in the winter (Gunkel 1939). Gunkel does not cite voucher material, but since a good photograph of a specimen is presented, we believe that its identification is correct. Kreisel (1996) characterized *P. impudicus* by a reticulate receptaculum surface, lack of veil, and a white pseudostipe, volva, and mycelial strand. Since the species is common in Europe, Gunkel (1939) theorized that spores had arrived around the 1930's with European boats that frequently moored at the port.

Clathrus columnatus was reported only once from Chile, based on a collection by C. Gay (Montagne 1853–54). Dring (1980) also reported collections from Valdivia, probably referring to the same collection. We were able to analyze Gay's collection (Herbarium P), but as the specimen is not well preserved, it was difficult to verify its identification. As previously discussed, Santesson (1943) suggested that Chilean specimens recorded as *Laternea triscapa* might also represent this species. *Clathrus columnatus* is reported from America, Africa, and Australasia, so its occurrence in Chile is expected. The species is characterized by 2–5 robust, spongy, reddish to orange columns free at the base and fused at the apex. The gleba is spread in the internal portion of the columns and is not confined to a glebifer.

Cunningham (1868, 1871) reported a *Clathrus* or *Ileodictyon* species found on sand in Chiloé Island (Quemchi). The material was later examined by Fischer (1893) and Dring (1980), who identified it as *Ileodictyon cibarium* Tul. & C. Tul. [≡ *Clathrus cibarius* (Tul. & C. Tul.) E. Fisch.]. Cunningham's collections are probably the ones mentioned by Lloyd (1909). Spegazzini (1921) reported the species as *Clathrus cibarius* (which he considered a variety of *C. gracilis*), growing on soil under *Persea lingue* (Miers ex Bertero) Nees. *Ileodictyon cibarium* was also reported by Parra & Escudero (1994) from the Region of Valparaiso. Parra & Escudero (1994) also reported *Clathrus* sp. from Cerro Pajonal, but their descriptions and illustrations lead us to conclude that their "*Clathrus* sp." also represents *I. cibarium*. Lazo et al. (1977) reported *Clathrus* spp. in exotic plantations near Peñuelas Lake, and Lazo (1983, 2001) reported *C. cibarium* (mistakenly published as *C. gracilis,* W. Lazo, pers. comm.) in Pumanque.

Ileodictyon gracile Berk. was reported from Chile many times (Montagne 1853–54, Léveillé 1846, Johow 1896, Philippi 1869). Montagne (1853–54) clearly stated that he had no voucher of *I. gracile* in his fungal collection and that his description was based on Gay's illustration and field notes. However, Léveillé (1846) stated that in Herbarium P there was a Chilean collection of *I. gracile* made by Gay. Fischer (1891) identified this collection as *I. cibarium;* our examination of Gay's collection at P confirms Fischer's identification.

Singer (1969) recorded *I. gracile* in Juan Fernández Archipelago and southern Chile, stating that it is easily recognized and the only clathroid species native to the region. However, our observation of a single glebifer in Singer's collection (#M7200, SGO) suggests that *I. gracile* probably represents a *Laternea* species.

Ileodictyon cibarium is similar enough to *I. gracile* that many authors had difficulties in separating them (e.g., Fischer 1890). The two species can be differentiated by the morphology of the receptacle arms (Dring 1980, Cunningham 1944): in *I. cibarium* the arms are thicker, concertina-like, and not noticeably thickened where anastomosed. The difference between the two species was well illustrated by Lloyd (1909) and Dring (1980). The original description of *I. gracile* (Berkeley 1845) shows a very clear illustration of the type, which allows us to differentiate it from *I. cibarium*. Basidiomata of *I. cibarium* are usually larger and may even reach a diameter of about 40 cm (Lloyd 1910).

Due to the great similarity between the two species and erroneous interpretations of *Ileodictyon* species, many Chilean records of the genus are misidentifications. The descriptions and illustrations presented in literature lead us to conclude that *I. cibarium* is the only *Ileodictyon* species definitely known to occur in Chile.

Key to the phalloid species known to occur in Chile

1. Basidiome hypogeous, sequestrate (truffle-like); exoperidium thick, yellowish to brownish. ..... ***Gelopellis macrospora***
1. Basidiome epigeous when mature, expanded; exoperidium thin, whitish to pale yellow. 2
2. Pseudostipe simple, cylindrical. 3
2. Pseudostipe absent; receptacle clathroid or columnar. 5
3. Receptacle conical. ..... ***Phallus impudicus***
3. Receptacle sphaerical, clathroid or surmounted by short arms 4
4. Pseudostipe surmounted by short arms. ..... ***Lysurus cruciatus***
4. Pseudostipe bearing an apical clathroid receptacle ..... ***Lysurus periphragmoides***
5. Receptacle clathroid, whitish; becoming detachable from the volva ..... ***Ileodictyon cibarium***
5. Receptacle ramified into columns, orange, pink to pale red; remaining attached to the volva. 6
6. Columns massive, reddish to orange, externally smooth; glebal mass spread internally in the arms; glebifer absent. ..... ***Clathrus columnatus***
6. Columns delicate, pinkish to pale red, externally with delicate crests; glebal mass confined to a single glebifer ..... ***Laternea pusilla***

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New records of cercosporoid fungi from Poland

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Abstract — Collections of four cercosporoid species are reported for the first time from Poland: *Cercospora echii* on *Echium vulgare*, *C. moravica* on *Caltha palustris*, *C. viburnicola* on *Viburnum opulus*, and *Passalora pastinacae* on *Pastinaca sativa*. Descriptions and illustrations of the fungi concerned are presented and remarks containing comparison with related species, distribution, and habitat are provided.

Key words — anamorphic fungi, hyphomycetes, leaf spot pathogens

Introduction

Cercospora Fresen. and *Passalora* Fr. are anamorphs of the ascomycetous genus *Mycosphaerella* Johanson (Shin & Kim 2001, Crous & Braun 2003, Crous 2009) belonging to the so-called cercosporoid fungi. Species of these genera are usually phytopathogenic, often causing leaf spots, occasionally hyperparasitic, and rarely saprobic (Hsieh & Goh 1990, Crous & Braun 2003, Agrios 2005, To-Anun et al. 2011).

Investigations of cercosporoid fungi from Poland have been carried out in the recent years, based on studies of field collections and revision of herbarium material. Thirty-eight *Cercospora* and thirty-one *Passalora* species have been registered in Poland (Świderska-Burek 2012). The results will be presented in a planned monograph of cercosporoid fungi of Poland.

Materials & methods

All collections examined are from Poland. The oldest material treated in this paper was collected in 1926 and is deposited in the herbarium of University of Warsaw. The other collections, deposited in the herbarium of the Department of Botany and Mycology in Lublin, are younger and have recently been re-examined.

The host plants infected with cercosporoid fungi were collected primarily from grasslands, taken to the laboratory, air-dried, and examined by light microscopy. The material was mounted in cotton blue and gently heated.

The monographs of Crous & Braun (2003), Chupp (1954), and Braun (1995) were used to identify the fungi. Nomenclature of the host plants follows The Plant List (http://www.theplantlist.org). Geographic regions of Poland follow the division used in Kondracki (2009). The specimens examined are deposited in the herbarium of the Department of Botany and Mycology in Lublin (LBL M) and Faculty of Biology in Warsaw (WA).

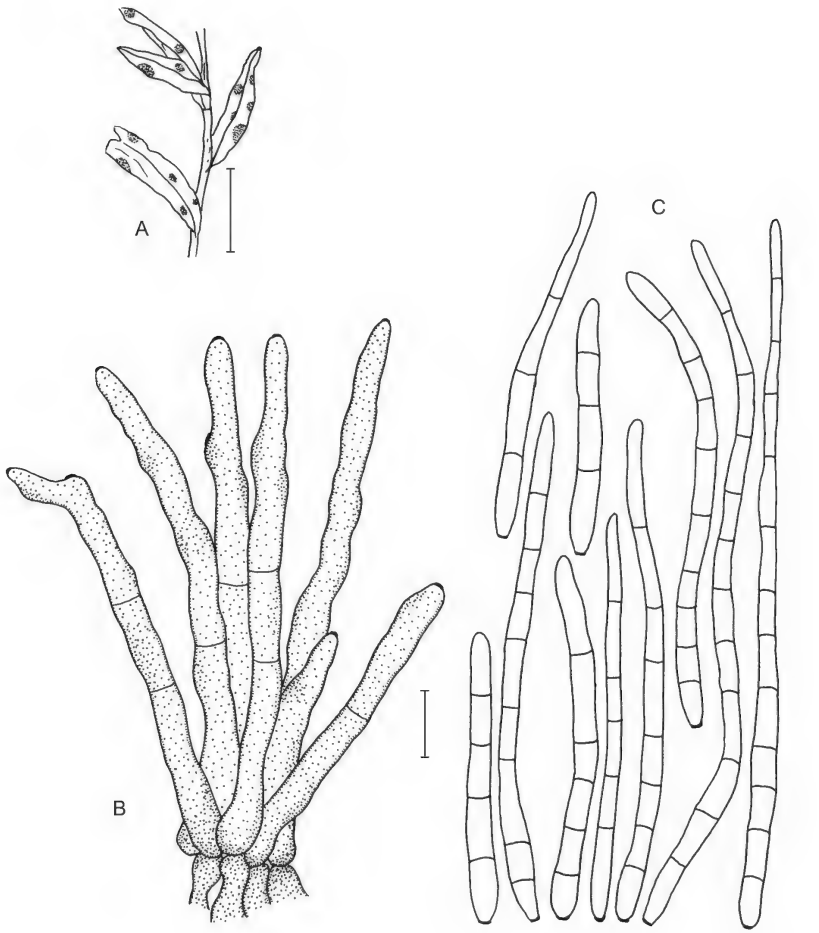

Fig. 1. *Cercospora echii* (LBL M–12168) on *Echium vulgare*.
A: Leaf spots. B: Conidiophores. C: Conidia.
Scale bars: A = 20 mm; B, C = 10 µm. U. Świderska-Burek del.

Taxonomy

Cercospora echii G. Winter, Hedwigia 23:190. 1884. FIG. 1

Leaf spots circular, 2–4 mm in diam., dark purplish or almost black center with pale margin. Fruiting amphigenous, mostly epiphyllous; stromata none or composed of several brown cells. Conidiophores 1–8 in fascicles, olivaceous-brown, multiseptate, rarely branched, up to 5 times geniculate, 40–142 × 4–5 µm. Conidia hyaline, acicular, straight or slightly curved, 3–12-septate, 40–125 × 2.5–3.5(–4) µm.

SPECIMEN EXAMINED — **POLAND. WOŁYŃSKA AND MAŁE POLESIE UPLAND**, Czumów village near Hrubieszów town, xerothermic slope, on *Echium vulgare* L. (*Boraginaceae*), 19 September 1995, A. Zając (LBL M–12168).

REMARKS: The fungus has been reported on four species of *Echium* — *E. italicum*, *E. platagineum*, *E. tuberculatum,* and *E. vulgare* — from Europe, Asia, Africa, North America, and Australia. *Echium vulgare* is distributed in Europe and southwestern Asia (Meusel et al. 1965–92, Zając & Zając 2009). In Europe, *C. echii* is known from five countries (Bulgaria, Portugal, Russia, Spain, and Ukraine; Crous & Braun 2003, Farr & Rossman 2014).

Cercospora echiorum is also known from *Echium* species, but it has shorter and slightly wider conidiophores (15–30 × 4–6 µm) and conidia (20–55 × 4–5 µm) (Chupp 1954). However, only *C. echii* has been found on *Echium vulgare* (Crous & Braun 2003).

Cercospora moravica (Petr.) U. Braun, Cryptog. Bot. 3: 235. 1993. FIG. 2

Leaf spots amphigenous, brown, angular, irregular or subcircular, 1–8 mm in diam., often confluent, margin indefinite, partially vein-limited. Fruiting amphigenous, whitish, sub-effuse. Conidiophores in loose or dense fascicles, hyaline, subcylindrical or geniculate-sinuous, straight, usually aseptate, 5.5–25 × 2–4 µm. Conidia hyaline, acicular, 1–8-septate, 30–135 × 1–2.5 µm.

SPECIMENS EXAMINED — **POLAND. NIZINA ŚRODKOWOMAZOWIECKA LOWLAND**, Garwolin town, water-meadow, on *Caltha palustris* L. (*Ranunculaceae*), 28 August 1986, H. Maszkiewicz (LBL M–12169); **NIZINA POŁUDNIOWOPODLASKA LOWLAND**, Firlej village near Lublin town, water-meadow, on *Caltha palustris*, 12 May 1989, E. Kasińska (LBL M–12170).

REMARKS: This fungus is confined to *Caltha palustris* and recorded only from Europe, although its host species is found also in North America and north Eurasia (Meusel et al. 1965–92, Zając & Zając 2009). *Cercospora moravica* has been collected in the Czech Republic, Finland, France, Germany, Great Britain, and Russia on this host (Crous & Braun 2003; Farr & Rossman 2014).

Cercospora palustris Losa was described on *Caltha palustris* from Spain in 1944, but this is an unresolved name (Crous & Braun 2003).

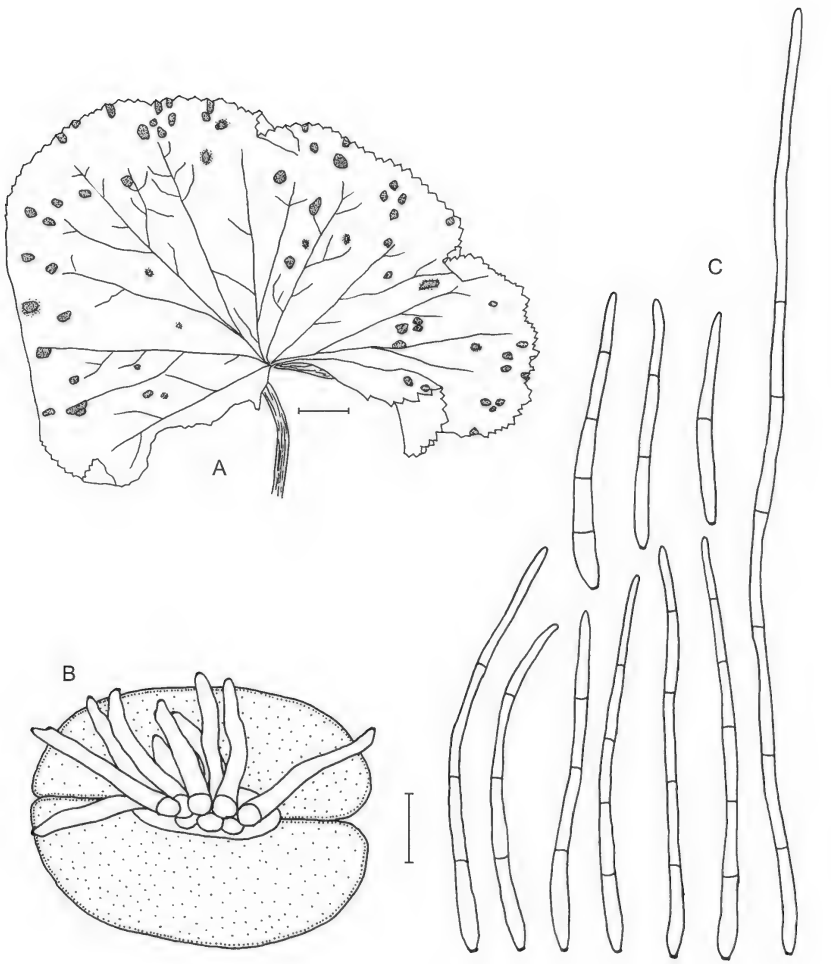

Fig. 2. *Cercospora moravica* (LBL M–12170) on *Caltha palustris*.
A: Leaf spots. B: Conidiophores. C: Conidia.
Scale bars: A = 20 mm; B, C = 10 µm. U. Świderska-Burek del.

Cercospora viburnicola W.W. Ray, Mycologia 33: 174. 1941. Fig. 3

Leaf spots angular or irregular, grayish brown with reddish brown margin. Fruiting amphigenous. Conidiophores mostly in dense fascicles, pale olivaceous-brown, straight, slightly curved or sinuous, sparingly septate,

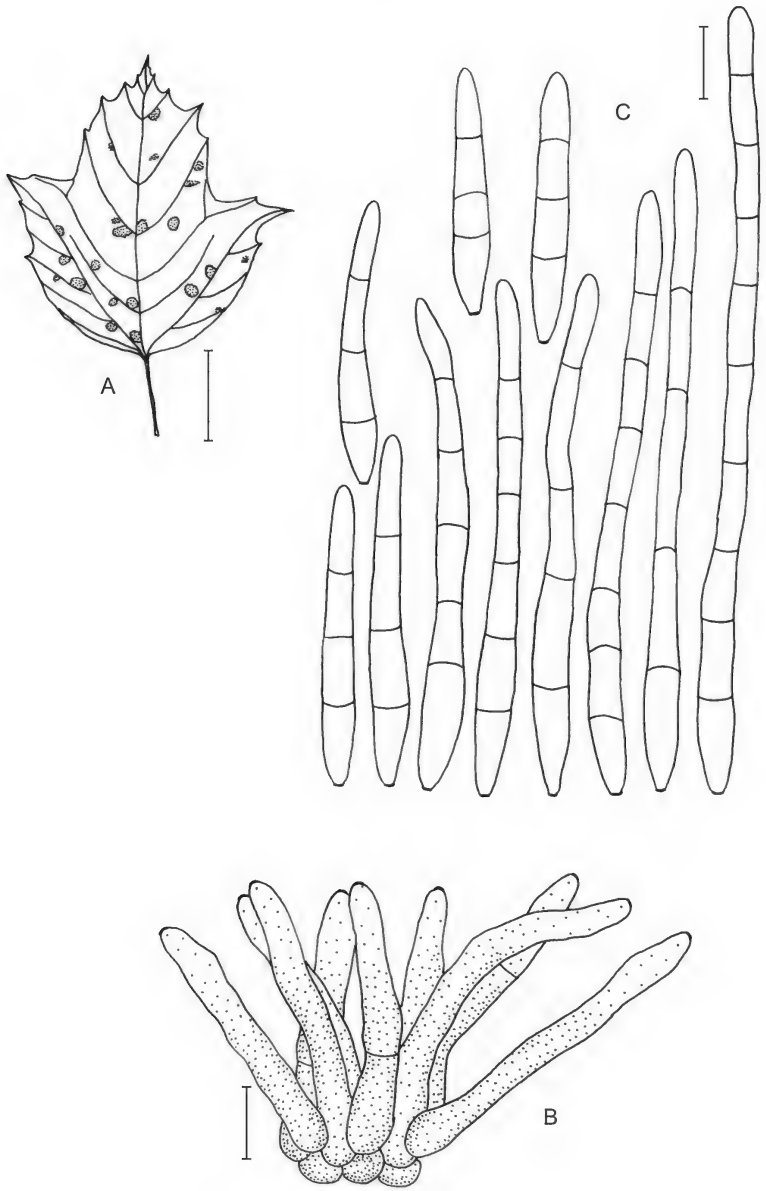

Fig. 3. *Cercospora viburnicola* (WA 029863) on *Viburnum opulus*.
A: Leaf spots. B: Conidiophores. C: Conidia.
Scale bars: A = 20 mm; B, C = 10 µm. U. Świderska-Burek del.

25–60 × 4–5.5(–6) µm. Conidia hyaline, acicular or obclavate, straight or slightly curved, 3–11-septate, 30–90(–205) × 3–5(–5.5) µm.

Specimen examined — **POLAND. Pojezierze Południowopomorskie Lakeland**, Jezioro Kadzionka Lake near Bydgoszcz town, on *Viburnum opulus* L. (*Adoxaceae*), 25 September 1926, N.N. (WA 029863, as *Cercospora* sp. on *Clematis vitalba*).

Remarks: This fungus has been recorded from only three countries — China, Korea, and USA — but from many localities and on many *Viburnum* spp. *Viburnum opulus* occurs in northwestern Eurasia (Meusel et al. 1965–92, Zając & Zając 2009). This is the first record of *C. viburnicola* in Europe (Crous & Braun 2003, Farr & Rossman 2014).

This species belongs to the complex of *Cercospora apii* s. lat. (Crous & Braun 2003). Environmental conditions, especially high temperature and humidity, influence the length of conidiophores and conidia. In the examined material, some conidia were longer (≤205 µm) and wider (usually 3–5.5 µm).

Six other cercosporoid hyphomycetes occur worldwide on *Viburnum*: *C. opuli* f. *brunneola* M.T. Lucas & Sousa da Câmara, *Passalora viburni* (Ellis & Everh.) U. Braun & Crous, *Pseudocercospora opuli* (Höhn.) U. Braun & Crous, *P. varia* (Peck) J.K. Bai & M.Y. Cheng, *P. viburni-cylindrici* (F.L. Tai) U. Braun, and *P. viburnigena* U. Braun & Crous. However, *C. viburnicola* is the only *Cercospora* species reported on *V. opulus* (Crous & Braun 2003).

Passalora pastinacae (Sacc.) U. Braun, Nova Hedwigia 55: 213. 1992. Fig. 4

Leaf spots angular, vein-limited, 1–3 mm in diam., mostly yellowish green or dark brown. Conidiophores solitary or 2–11 in fascicles, pale olivaceous, olivaceous-brown or yellowish brown, usually 1–2 geniculate, septate, not branched, 12–48 × 5–7 µm. Conidia subhyaline, cylindrical to obclavate, straight or slightly curved, 1–5-septate, 25–75 × 4–7 µm.

Specimen examined: **POLAND. Niecka Nidziańska Basin**, Rezerwat Skorocice reserve near Busko-Zdrój town, on *Pastinaca sativa* L. (*Apiaceae*), 14 September 1979, J. Romaszewska-Sałata (LBL M–12171).

Remarks: This fungus is known only from *Pastinaca sativa*; outside Europe it has been reported from Africa, North America, and Australia. According to Meusel et al. (1965–92) and Zając & Zając (2009), the host plant commonly occurs in semi-natural and synanthropic (transformed) habitats in Europe, but *P. pastinacae* has been found only in four European countries — Austria, Italy, Russia, and Romania (Crous & Braun 2003, Farr & Rossman 2014).

Cercospora apii is the only other cercosporoid species known to occur on *Pastinaca*. *Passolora pastinacae* differs from *C. apii* in having shorter, cylindrical–obclavate, not quite colourless conidia (Chupp 1954, Crous & Braun 2003).

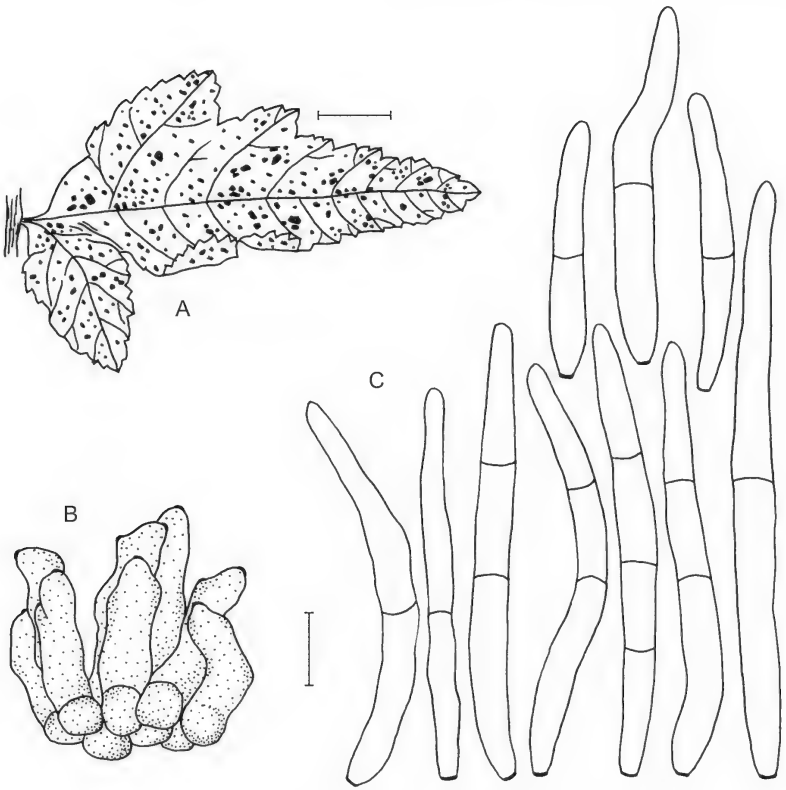

Fig. 4. *Passalora pastinacae* (LBL M–12171) on *Pastinaca sativa*.
A: Leaf spots. B: Conidiophores. C: Conidia.
Scale bars: A = 20 mm; B, C = 10 µm. U. Świderska-Burek del.

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New records of *Phragmidium* species from Pakistan

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Abstract — Five *Phragmidium* species on different rosaceous hosts are described and illustrated. *Phragmidium violaceum* is a new record for Pakistan. Aecidial stages of *P. mucronatum*, *P. rubi-idaei*, and *P. tuberculatum* are re-described following study of their wall characters with SEM. *Potentilla gerardiana* represents a new host record for *Phragmidium papillatum*.

Key words — *Caeoma*, Khanspur, Mansehra, *Phragmidiaceae*

Introduction

The *Phragmidiaceae* are a family of rust fungi in the order *Pucciniales*. Cummins & Hiratsuka (2003) reported that this family contains 14 genera and 164 species. Its genera are distinguished by their aecial, uredinial, and telial stages. Species of *Phragmidium* Link typically infect plant species in the *Rosaceae*. Their telia contain teliospores borne singly on often hygroscopic pedicels and with smooth or (more often) verrucose walls and 1- to several transversely septate cells with 2–3 pores in each cell. The aecia are usually *Caeoma*-type (with catenulate spores) or less often *Uredo*-type (with spores borne singly). About 15 *Phragmidium* species have been described or reported from Pakistan (Ahmad et al. 1997; Afshan et al. 2011, 2012). In this paper, we describe and illustrate five *Phragmidium* species and provide SEM photographs to illustrate important aeciospore wall characters.

Materials & methods

Freehand sections of infected tissue and spores were mounted in lactophenol and gently heated to boiling point. The preparations were observed under a Nikon YS 100 microscope and photographed with a Digipro-Labomed and a JSM5910 scanning electron microscope. Spores and paraphyses were drawn using a Camera Lucida (Ernst Leitz Wetzlar, Germany). Spores were measured using an ocular micrometer, with at

least 25 spores measured for each spore stage. The rusted specimens have been deposited in the herbarium of the Botany Department, University of the Punjab, Lahore (LAH).

Taxonomy

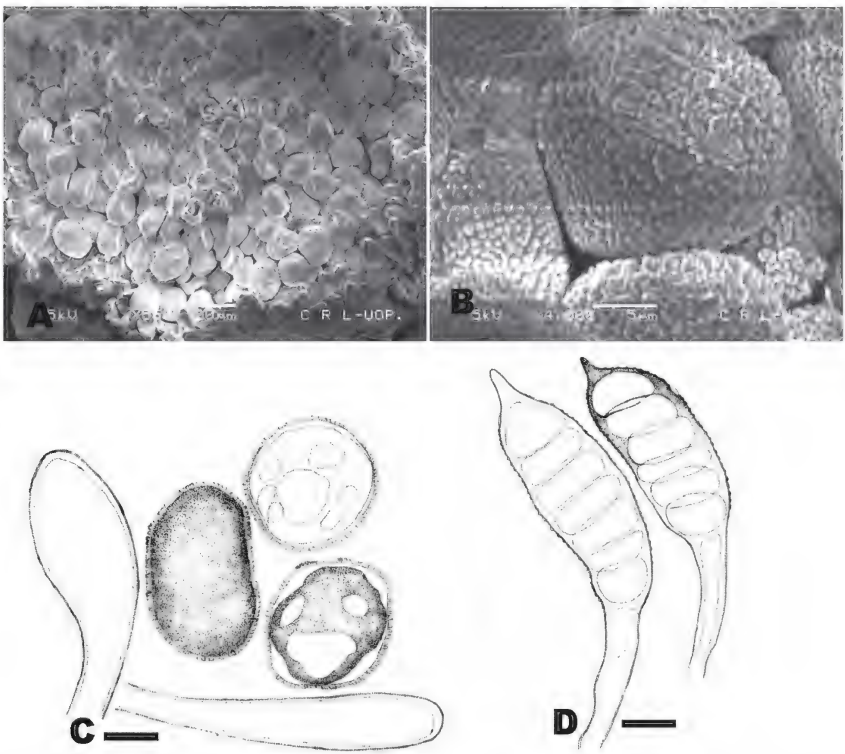

Plate 1: *Phragmidium mucronatum*. (A) SEM photograph of an aecium showing aeciospores surrounded by slightly incurved to erect paraphyses. (B) SEM photograph of aeciospores showing verrucose wall ornamentation. (C) Aeciospores with paraphyses. (D) Teliospores. Scale bars: C, D = 10 µm.

Phragmidium mucronatum (Pers.) Schltdl., Fl. berol. 2: 156 (1824) Plate 1

Spermogonia not found. Aecia hypophyllous, on leaves, bright yellow to orange yellow, rounded or oblong, scattered, sori 0.1–0.4 × 0.09–0.3 mm; surrounded by paraphyses; paraphyses numerous, slightly incurved to erect, clavate, hyaline to nearly colorless, 50–70 µm long and 12–15 µm wide. Aeciospores globose–subglobose or ellipsoid, 16–28 × 23–32 µm (mean 19 × 27.7 µm); wall 1.5–2 µm thick, with obscure, scattered pores, hyaline with yellowish orange to dark orange contents, densely and distinctly verrucose. Uredinia hypophyllous, scattered, yellow; urediniospores rare and not very

clear; paraphyses curved, clavate. Telia hypophyllous, scattered, minute, black. Teliospores cylindrical to ellipsoid or oblong, 3–8 celled, 27–32 × 57–110 µm; wall 4–7 µm thick, verrucose, dark brown to chestnut brown; apex conical, 5–10 µm thick; pedicel long, hyaline to pale yellow.

Material examined: **PAKISTAN**, **Khyber-Pakhtunkhawah**, Khaira Gali, 2347 m a.s.l., on *Rosa macrophylla* Lindl., stages I + II + III, 19 June 2008, NSA #150 (LAH NSA1022).

Comments: *Phragmidium mucronatum*, cosmopolitan on different *Rosa* species (Wilson & Henderson 1966; Hiratsuka et al. 1992), was previously reported from Pakistan on *Rosa* sp. from Rawalpindi (Ahmad 1956a,b).

Phragmidium papillatum Dietel, Hedwigia 29: 25 (1890) Plate 2

Spermogonia and aecia not found. Uredinia mostly hypophyllous on leaves, or on the veins and petioles, bright yellow to orange, scattered, rounded

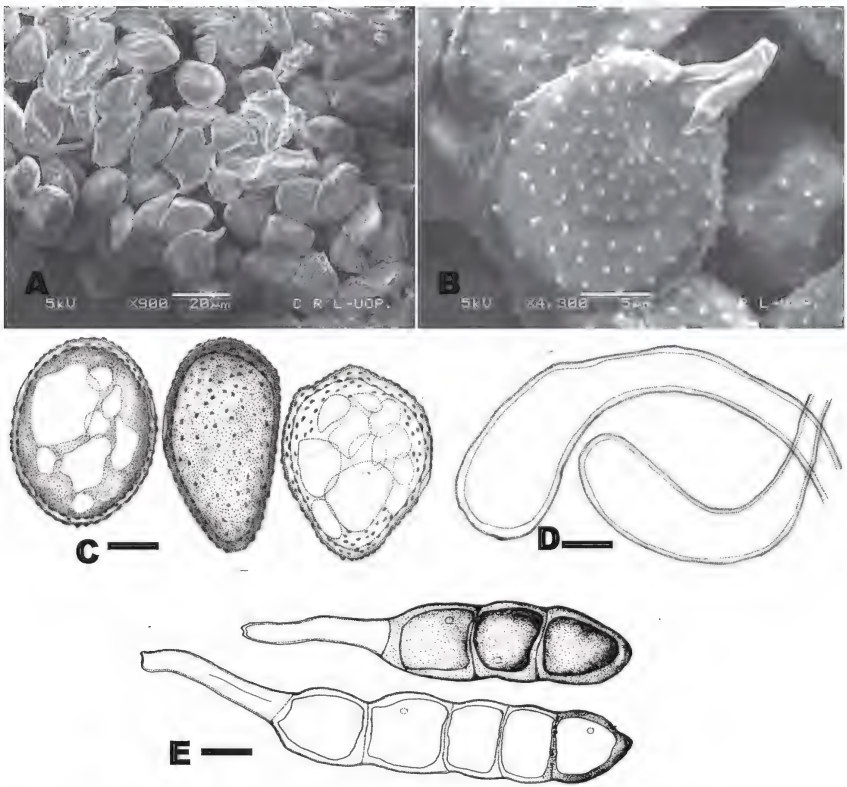

Plate 2: *Phragmidium papillatum*. (A) SEM photograph of uredinium containing urediniospores. (B) A urediniospore showing echinulate to verrucose wall ornamentation. (C) Urediniospores. (D) Paraphyses (E) Teliospores. Scale bars: C–E = 10 µm.

or oblong, sori 0.09–0.2 × 0.2–0.5 mm; PARAPHYSES numerous, incurved to suberect, clavate, hyaline to nearly or quite colorless, 50–80 µm long and 11–15 µm wide. UREDINIOSPORES globose-subglobose or ellipsoid to ovoid, 16–22 × (19–) 21–32 µm (mean 19.0 × 24.5 µm); wall 1–1.5 µm thick, with 2–5 scattered pores, yellow to yellowish orange, echinulate to verrucose, with a hyaline pedicel. TELIA hypophyllous, scattered or gregarious, minute, rounded or irregular, black. TELIOSPORES cylindrical or ellipsoid to oblong, 3–5 celled, rarely 2-celled, 28–34 × 57–95 µm, slightly or not constricted at the septum; wall 2–5 µm thick, smooth, brown to yellowish brown; pedicel long, 6–8 × 30–95 µm, hyaline to pale yellow.

MATERIAL EXAMINED: **PAKISTAN, AZAD JAMMU & KASHMIR**, Neelum valley, Muchal, 3000 m a.s.l., on *Potentilla gerardiana* Lindl., stage II, 3 Nov. 2006, NSA #30906 (LAH NSA1024a); **KHYBER-PAKHTUNKHAWAH**, Mansehra, Oghi, 1122 m a.s.l., on *Potentilla gerardiana*, stages II + III, 16 May 2008, NSA #160408 (LAH NSA1024b).

COMMENTS: *Phragmidium papillatum* has been reported on *Potentilla cryptotaeniae* Maxim. and *P. matsumurae* Th. Wolf from Japan and is also common in USSR, China, and Korea (Hiratsuka et al. 1992). From Pakistan, it has previously been reported on *Potentilla nepalensis* Hook. from Kalam (Ahmad 1956a,b), Swat valley (Ono & Kakishima 1992), Kaghan valley (Ono 1992), and Murree (Kakishima et al. 1993a,b). *Potentilla gerardiana* is a new host for this rust fungus.

Phragmidium rubi-idaei (DC.) P. Karst., Bidr. Känn. Finl. Nat. Folk 31: 52 (1879)

PLATE 3

SPERMOGONIA and UREDINIA not found. AECIA hypophyllous, on leaves, solitary or in groups, bright yellow, scattered, sori 0.2–0.3 × 0.1–0.4 mm; surrounded by paraphyses, numerous, incurved to suberect, clavate, hyaline to nearly colorless, 50–70 µm long and 10–16 µm wide. AECIOSPORES globose–subglobose or ovoid to obovoid, 14–17 × 15–23 µm (mean 15.9 × 17.5 µm); wall 1–1.5 µm thick, hyaline to light yellow with yellowish orange contents, sparsely echinulate, echines produced on a distinct base. TELIA hypophyllous, scattered, naked, black. TELIOSPORES cylindrical, 4–10 celled, 28–35 × 70–122 µm, slightly or not constricted at the septum; wall 2–5 µm thick, verrucose, yellowish brown to chestnut brown; apex rounded or attenuated, 10–15 µm long, hyaline; pedicel long, 10–15 × 80–150 µm, hyaline to pale yellow.

MATERIAL EXAMINED: **PAKISTAN, KHYBER-PAKHTUNKHAWAH**, Khaira Gali, 2347 m a.s.l., on *Rubus niveus* Thunb., stages I + III, 19 Jun. 2008, NSA #153 (LAH NSA1026).

COMMENTS: *Phragmidium rubi-idaei* is cosmopolitan in temperate regions on various *Rubus* species (Wilson & Henderson 1966; Hiratsuka et al. 1992). It has previously been reported from Pakistan on *Rubus idaeus* L. from Khanspur

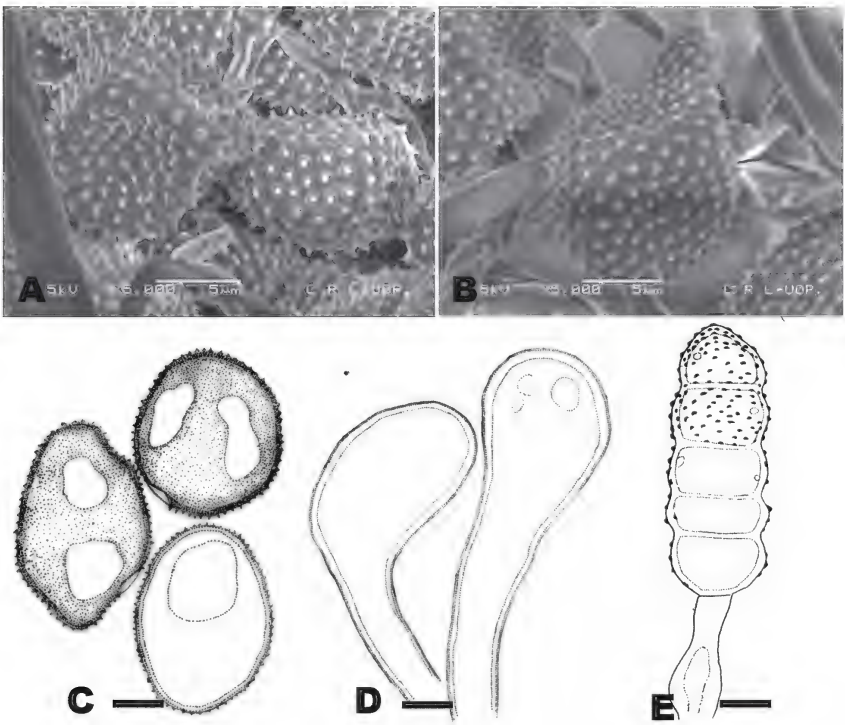

Plate 3: *Phragmidium rubi-idaei.* (A) SEM photograph of aeciospores showing echinulate wall ornamentation. (B) A closer view of an aeciospore. (C) Aeciospores. (D) Paraphysis (E) Teliospores. Scale bars: C–E = 10 µm.

(Khalid et al. 1993). The aecidial stage of *P. rubi-idaei* represents a new record for Pakistan.

Phragmidium tuberculatum Jul. Müll., Ber. dt. bot. Ges. 3: 391 (1885) Plate 4

Spermogonia and Uredinia not found. Aecia hypophyllous, on branches, petioles and leaves, bright yellow to orangish yellow, rounded or oblong, scattered, sori 0.1–0.3 × 0.09–0.1 mm; paraphyses numerous, incurved to suberect, clavate or clavate to capitate, hyaline to nearly or quite colorless, 55–70 µm long and 8–10 µm wide, with apex 15–20 µm in diameter. Aeciospores globose to subglobose or ellipsoid, 18–22 × 21–27 µm; wall 1–1.5 µm thick, with 5–8 scattered pores, hyaline with orange–yellow contents, densely verrucose, one or shorter, cylindrical verrucae produced on a distinct base. Telia hypophyllous, scattered, minute, black. Teliospores ellipsoid to cylindrical, 3–7-celled; 25–30 × 57–85 µm; wall 3–5 µm thick, verrucose, dark brown to chestnut brown; pedicel long, hyaline to pale yellow.

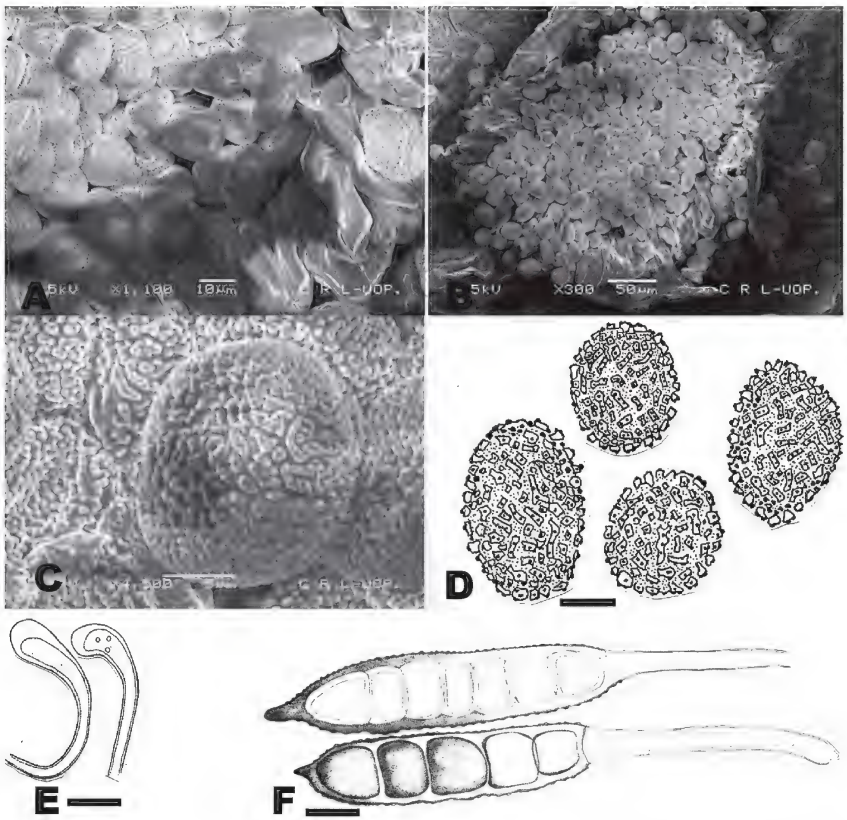

Plate 4: *Phragmidium tuberculatum*. (A) SEM photograph of an aecium. (B) Aeciospores with incurved paraphyses. (C) A close view of surface ornamentation of aeciospores showing cylindrical verrucae produced on a distinct base. (D) Aeciospores. (E) Paraphyses. (F) Multicelled teliospores. Scale bars: D–F= 10 µm.

Material examined: **PAKISTAN**, **Khyber-Pakhtunkhawah**, Ayubia National Park, 2135 m a.s.l., on *Rosa moschata* Herrm., stages I + III, 24 May 2006. NSA #04 (LAH NSA1028).

Comments: *Phragmidium tuberculatum* is cosmopolitan and reported on different *Rosa* species (Wilson & Henderson 1966; Farr & Rossman 2014); it has previously been reported from Pakistan on *Rosa webbiana* Wall. ex Royle and *Rosa* sp. from Kaghan valley, Barum valley, Dungagali, and Swat valley (Ahmad 1956a,b; Kakishima et al. 1993a,b: Okane et al. 1992; Ono & Kakishima 1992).

Phragmidium violaceum (Schultz) G. Winter, Hedwigia 19: 54 (1880) Plate 5

Spermogonia, uredinia and telia not found. Aecia amphigenous, yellowish orange, scattered, on veins and petioles, sori 0.5 × 0.09–0.1 mm.

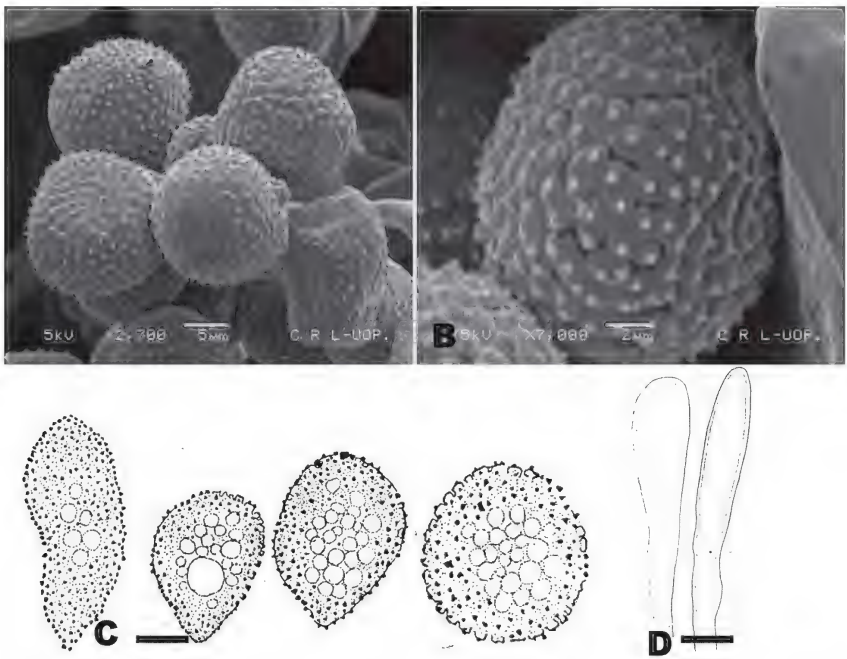

Plate 5: *Phragmidium violaceum*. (A) SEM photograph of aeciospores. (B) A closer view of an aeciospore showing verrucose wall ornamentation. (C) Aeciospores. (D) Paraphyses. Scale bars: C = 10 µm; D = 22 µm.

Aeciospores globose to ovoid or ellipsoid; wall aculeate–verrucose to echinulate, verrucae produced on a distinct base, yellow to orange yellow, sometimes with orangish yellow granules; 16–24 × 20–33 µm; surrounded by peripheral, straight or incurved, clavate and hyaline paraphyses, 94–118 µm long, 8–19µm thick, with uniformly thin walls.

Material examined: **PAKISTAN, Azad Jammu & Kashmir**, Neelum valley, Muchal, 3000 m a.s.l., on *Rubus ulmifolius* Schott, stage I, 3 Nov. 2006, NSA #0101 (LAH NSA1029a); **Khyber-Pakhtunkhawah**, Khanspur, 2135 m a.s l., on *Rubus ulmifolius*, stage I, 24 May 2006, NSA #01 (LAH NSA1029b).

Comments: *Phragmidium violaceum* is widespread in Britain, Ireland, North America, Europe, and Africa on different *Rubus* species (Wilson & Henderson 1966; Laundon 1969) and has been introduced as a biological control agent in Australia, New Zealand, and Chile. This report represents a new record for Pakistan.

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Life cycle of *Aecidium araliae* and its new name

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Abstract — Field observations and inoculation experiments with the uredinial and telial stages of a rust on *Carex stenostachys* var. *cuneata* clarified that these were conspecific with the spermagonial and aecial stages of *Aecidium araliae* on *Aralia elata*. Based on uredinial and telial morphologies *A. araliae* is transferred to *Puccinia* as *P. caricis-araliae* nom. nov.

Key words — *Araliaceae, Cyperaceae, Pucciniaceae,* rust fungus, taxonomy

Introduction

Aecidium araliae, initially published invalidly (Sawada 1943: lacking a Latin description), was subsequently validated by Ito & Murayama (1943). This rust fungus has been known to produce only spermogonial and aecial stages on plants within the *Araliaceae* and is widely distributed in Japan, Taiwan, and China: on *Aralia elata* (Miq.) Seem. in Japan (Ito & Murayama 1943, Ito 1950, Hiratsuka 1952, 1960, Kakishima et al. 1986, Harada 1994), Taiwan (Tai 1979, Hiratsuka & Chen 1991) and China (Tai 1979); on *A. decaisneana* Hance in Taiwan (Hiratsuka 1943, Ito & Murayama 1943, Sawada 1943, Ito 1950, Tai 1979, Hiratsuka & Chen 1991); on *A. chinensis* L. in China (Tai 1979); on *A. spinosa* L. in Taiwan (Hiratsuka & Chen 1991); on *Agalma lutchuense* Nakai [= *Schefflera heptaphylla* (L.) Frodin] in Japan (Hiratsuka 1952, 1960, Shimabukuro 1961, Hiratsuka et al. 1985); and on *Textoria trifida* (Thunb.) Nakai ex Honda [≡ *Dendropanax trifidus* (Thunb.) Makino ex Hara] in Japan (Hitatsuka 1952, 1960). Spermogonia and aecia were observed on leaves and petioles that were usually malformed and enlarged by the infections (Fig. 1A–D) with the surface of these infected tissues becoming yellow because of abundant aeciospore production.

In June 1992, the rust was observed in Yamagata Pref., Japan, in a field of *Aralia elata* cultivated to harvest its sprouts as an early spring vegetable (Fig. 1A,B). By midsummer the infection had almost disappeared. As other life stages of the rust could not be found, it was suspected that the rust was heteroecious and produced other stages on different host plants around these fields. We surveyed other rust fungi in the same vicinity and collected some candidates for this species. Inoculation experiments were carried out to confirm their connection. Finally, we found uredinial and telial stages on *Carex stenostachys* var. *cuneata* (Ohwi) Ohwi & T. Koyama and confirmed these were the other spore stages of this species. We present results of inoculation experiments and a taxonomic treatment of the species, based on morphology.

Materials & methods

Inoculations

Telia on dead leaves of *Carex stenostachys* var. *cuneata* were collected at Numasawa, Higashine-shi, Yamagata Pref., Japan on 30 April 1994 (Fig. 2A,B) and kept in a refrigerator at 5°C for use in inoculations. The leaves with telia were soaked in running tap water for several days to induce germination of teliospores (Fig. 2C). Once numerous basidiospores were produced from teliospores then inoculations to *A. elata* were carried out several times from May to June at University of Tsukuba, Tsukuba, Ibaraki Pref., Japan. For inoculation, leaves with germinating teliospores were cut into small pieces (ca. 5 mm^2) and placed on healthy leaves of *A. elata*. The *A. elata* plants were transferred from the field and grown in clay pods. The inoculated plants were kept in a moist chamber in darkness at about 20°C for 2 days and then transferred into a growth cabinet at about 20°C with artificial illumination for observations.

Morphological observations

Dry specimens collected in the field or obtained from inoculations were observed morphologically by light (LM) and scanning electron microscopy (SEM). The spores or thin sections of sori were mounted in lactophenol solution on glass slides for LM observations. For SEM, sori and spores obtained from specimens were attached to specimen holders by double-sided adhesive tape and coated with gold in high vacuum with an Eiko IB-3 Ion Coater. They were examined with a Hitachi S-430 SEM operated at 20kV. All specimens were deposited in the fungal herbarium of the Department of Botany, National Museum of Nature and Science, Tsukuba, Japan (TNS).

Results & discussion

Life cycle

Seven to eight days after inoculation with basidiospores produced by teliospores on *C. stenostachys* var. *cuneata,* small yellow spots of spermogonia appeared on the surface of leaves and stems of *A. elata*. The infected tissues were malformed and discolored. About 10 days later, cupulate aecia with aeciospores were produced on the same leaves and stems (Fig. 1C). The results

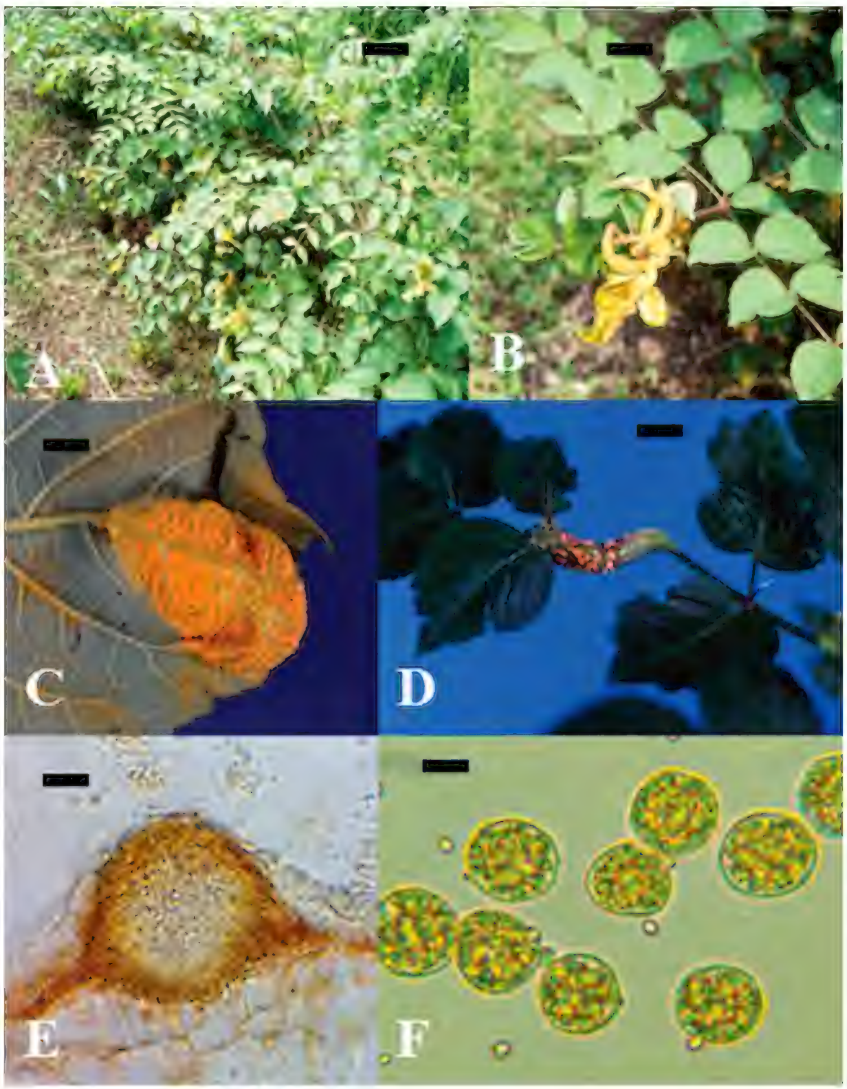

Figure 1. Spermogonial and aecial stages of *Puccinia caricis-araliae* on *Aralia elata*. A: Symptoms of *A. elata*. B: Aecia produced on malformed stems. C: Aecia with aeciospores. D: Aecia produced by the inoculation. E: Vertical section of a spermogonium. F: Aeciospores. Scale bars: A = 10 cm; B = 5 cm; C = 1 cm; D = 3 cm; E = 2 µm; F = 20 µm.

of inoculations confirmed that the telial stage on *C. stenostachys* var. *cuneata* and the aecial stage on *A. elata* are produced by the genetically same species.

TABLE 1. Comparison of *Puccinia* species on *Carex* with two germ pores at each urediniospore base.

Species	Urediniospores		Teliospores		Aecial hosts
	Size (μm)	Wall	Size (μm)	Apical thickness (μm)	
P. caricis-araliae [1]	17–25.5 × 16–24	Pale brown; echinulate	30.5–47 × 10–16.5	4–13	*Aralia elata* (*Araliaceae*)
P. kawakamiensis [2]	17–26 × 13–20	Brown; echinulate	32–55 × 10–18	5–18	*Circaea erubescens* (*Onagraceae*)
P. pulchella [3] (nom. illeg.)	20–32 × 15–23	Pale brown; echinulate	27–47 × 11–19	7–13	*Viola* spp. (*Violaceae*)
P. yokogurae [4]	21–29 × 16–21	Pale brown; echinulate	29–45 × 16–21	9–13	?
P. yokotensis [4]	20–26 × 18–21	Pale brown; echinulate	29–39 × 12–18	11–13	?

REFERENCES— [1] This paper; [2] Kakishima & Sato (1983); [3] Ono & Kakishima (1981); [4] Ito (1950)

Morphology

Spermogonia and aecia on *A. elata* produced by inoculations were morphologically similar with those of specimens collected in the field. As the morphological characteristics matched those previously described for *A. araliae* (Ito & Murayama 1943, Ito 1950), these spermogonial and aecial stages were identified as *A. araliae*.

Telial morphology and the two-celled teliospores confirm that the rust on *C. stenostachys* var. *cuneata* belongs to the genus *Puccinia* (Cummins & Hiratsuka 2003). Many *Puccinia* species have been reported on *Carex* spp. in Asia (Ito 1950, Tai 1979, Hiratsuka & Chen 1991, Hiratsuka et al. 1992). They are separated based on position of urediniospore germ pores, as teliospores are morphologically variable and similar to each other. The *Puccinia* on *C. stenostachys* var. *cuneata* collected in Yamagata Prefecture has two germ pores at the base of the urediniospores. It differs morphologically from four other species on *Carex* that have two basal germ pores (TABLE 1). Two of these species have recorded aecial hosts, but these are in *Onagraceae* and *Violaceae* (and not *Araliaceae*). *Puccinia* species producing spermogonia and aecia on *Aralia* have not been reported anywhere in the world. Morphological details of this species are given below.

Taxonomy

Its telial morphology supports the transfer of *A. araliae* to *Puccinia.* However, as the name *P. araliae* has already been published for a different species producing telia on *Aralia,* we propose a replacement name in *Puccinia* for *Aecidium araliae.*

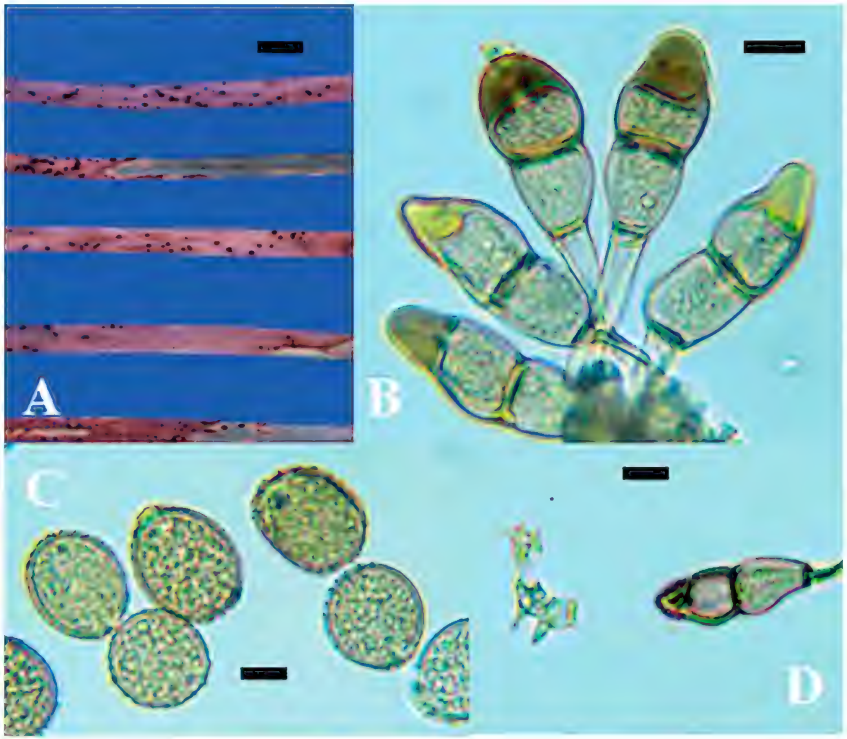

FIGURE 2. Uredinial and telial stages of *Puccinia caricis-araliae* on *Carex stenostachys* var. *cuneata*. A: Telia on *C. stenostachys* var. *cuneata*. B: Teliospores. C: Urediniospores. D: Germination of a teliospore. Scale bars: A = 5 cm; B, C = 10 µm; D = 15 µm.

Puccinia caricis-araliae Kakish. & Q. Wang, **nom. nov.** FIGS 1–3

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≡ *Aecidium araliae* Sawada ex S. Ito & Muray., Transactions of the Sapporo Natural History Society 17: 171, 1943, non *Puccinia araliae* Ellis & Everh. 1891.

TYPE: Japan, Yamagata Pref., Higashine-shi, on *Aralia elata*, stages 0, I, May 1992 (**Neotype designated here**, TNS-F-57726). Japan, Yamagata Pref., Higashine-shi, Numasawa, on *Carex stenostachys* var. *cuneata*, stages II, III, 30 April 1994 (**Epitype designated here**, TNS-F-57724).

"*Aecidium araliae*" Sawada, Journal of the Natural History Society Taiwan 33: 97, 1943, nom. inval. (no Latin).

SPERMOGONIA epiphyllous, surrounded by discolored lesions, yellow to brown, flask-shaped (type 7 of Cummins & Hiratsuka 2003). AECIA hypophyllous, densely grouped, cupulate with peridia, pale yellow. AECIOSPORES globose to subglobose, often angular, 18–35 × 16–24 µm, wall 2.0–2.5 µm thick, hyaline, verrucose with large granules. UREDINIA hypophyllous, scattered, erumpent,

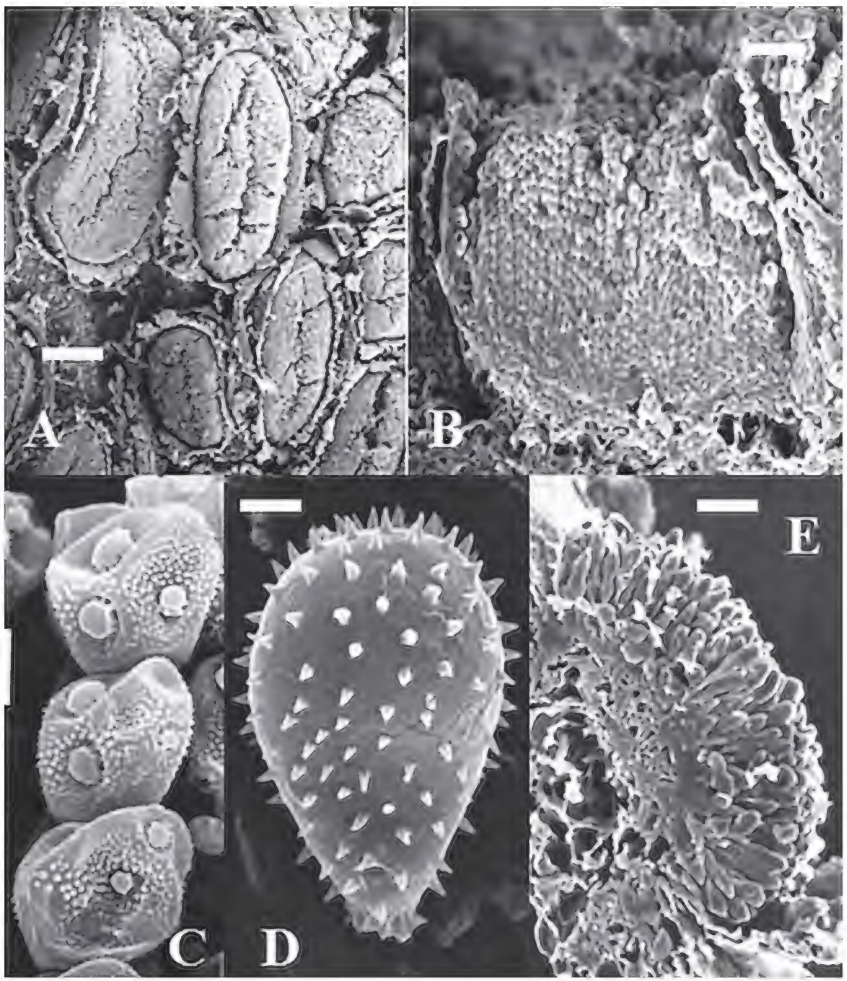

Figure 3. Morphology of *Puccinia caricis-araliae* observed by SEM. A: Aecia on *Aralia elata*. B: Vertical section of an aecium on *A. elata*. C: Aeciospores with large granules. D: Urediniospore. E: Vertical section of a telium on *Carex stenostachys* var. *cuneata*. Scale bars: A = 200 μm; B = 50 μm; C = 5 μm; D = 3 μm; E = 25 μm.

brown to pale brown. Urediniospores obovoid or broadly ellipsoid, 17–25.5 × 16–24 μm (av. 21.0 × 18.5 μm), wall ca. 1.5 μm thick, hyaline to pale brown, echinulate, germ pores 2 at base. Telia hypophyllous, scattered, ellipsoid, erumpent, dark brown. Teliospores clavate to oblong with round and obtuse apices, attenuate towards the base, constricted at the septa, 30.5–47 × 10–16.5 μm, wall pale brown to brown, thickened at apex (4–13 μm), smooth; pedicel short, persistent.

Other Specimens examined: On *Carex stenostachys* var. *cuneata*: JAPAN, Yamagata Pref., Higashine-shi, Numasawa, stage II, 18 June 1994 (TNS-F-57725).

On *Aralia elata*: JAPAN, Gunma Pref., Kanra-gun, Nanmoku-mura, stages 0, I, May 2002 (TNS-F-57728); Ibaraki Pref., Tsukuba-shi, stages 0, I, 18 May 1994 (TNS-F-57727); Tsukuba-shi, University of Tsukuba, stages 0, I, post-inoculation, 13 June 1994 (TNS-F-57729); stages 0, I, post-inoculation, 18 June 1994 (TNS-F-57730).

Comments: The type specimen of *A. araliae* was not designated when Ito & Murayama (1943) validated this species, although they described two *Aralia* species as host plants. Because their specimens also could not be found in the herbarium of Hokkaido University, Japan (SAPA), we have selected a neotype specimen for this species.

Spermogonial and aecial stages of this rust fungus were confirmed by inoculation only on *A. elata*. However, several species of *Araliaceae* have been reported as host plants of *A. araliae,* which is widely distributed in Japan, China, and Taiwan. Therefore, we suspect that other *Carex* species also serve as uredinial and telial hosts because *C. stenostachys* var. *cuneata* is mainly distributed in northern area of Honshu Island of Japan.

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Two rare myxomycete species from the Iberian Peninsula

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Abstract — Two uncommon myxomycete species, *Diderma sauteri* and *Stemonitopsis microspora*, recently recorded in the Iberian Peninsula, are described, illustrated, and discussed.

Key words — *Amoebozoa*, *Physarales*, *Stemonitales*, taxonomy, distribution

Introduction

As a result of a fungal and myxomycete inventory of the 'Río Caselas' region, located in the Northwest of Spain, Pontevedra province (Requejo 2013; Requejo & Andrés Rodríguez 2013), two interesting taxa were obtained. After a detailed morphological study, these collections were identified as *Diderma sauteri* and *Stemonitopsis microspora,* two species that are rare in southwestern Europe and which are described and discussed here.

Materials & methods

The collection and study of the specimens was made following the usual methodology for myxomycetes (Martin & Alexopoulos 1969; Nannenga-Bremekamp 1991). Macroscopic characters were studied using a stereomicroscopic Nikon model SMZ745T. Microscopic observations were made using a Nikon E100 microscope. Samples were prepared in KOH (5–10%). For measurements and microscopic images of the structures we used the software ProgRes® CapturePro 2.7, associated with the digital camera Jenoptic CT3 (Jenoptik AG, Germany).

The studied collections are deposited in the personal herbarium of one of the authors (req-Fungi) with duplicates in Real Jardín Botánico, C.S.I.C. (MA-Fungi).

Taxonomy

Diderma sauteri (Rostaf.) T. Macbr., N. Amer. Slime-moulds:103.1899 PLATE 1·1–2

SPOROCARPS gregarious or scattered, sessile, globose to subglobose, sometimes pulvinate, with flattened areas by mutual pressure, up to 1 mm in diameter. SPOROTHECA pink, grey, pinkish-brown or brownish. HYPOTHALLUS undetectable. PERIDIUM double, the external layer, cartilaginous, thin, smooth, separate from the inner; inner layer membranous, grey or iridescent, with amorphous calcium carbonate granules of 1–3 μm diameter. COLUMELLA rudimentary, calcareous, often reduced to a reddish-brown basal thickening. CAPILLITIUM filamentous, limeless, smooth, colourless to violet, branched and few anastomosed. SPORES free, black in mass, dark violet-brown in transmitted light by LM, globose, (n = 62) (11.2–)11.8–15.0(–15.2) μm, spinulose.

SPECIMENS EXAMINED — **SPAIN**: **PONTEVEDRA**. Salceda de Caselas, A Laxe, Río Caselas, 29TNG3676, 90 m, on bryophytes, 10-I-2012, leg. Óscar Requejo (req-Fungi 423; MA-Fungi 86674).

COMMENTS — This collection fits well the *D. sauteri* descriptions of Poulain et al. (2011), Ing (1999), and Neubert et al. (1995), although we have recorded a slightly wider range of spore sizes. Our observations of abnormally large spores up to 21 μm diam. coincide with those of Martin & Alexopoulos (1969); these authors mentioned the possibility of such abnormalities when there is an anomalous sporocarp development, and therefore we place no taxonomic importance on such features.

Diderma sauteri is usually associated with bryophytes. The most similar species is *Diderma ochraceum* Hoffm., which has smaller spores and bright orange, red, or yellow sporothecas (Poulain et al. 2011). *Diderma subincarnatum* Kowalski shares with *D. sauteri* the pinkish colors of the sporotheca but the spores are slightly larger (13–16 μm diam) and usually grow in dead branches and leaves (Poulain et al. 2011).

This is the first confirmed record of *D. sauteri* in Spain. We know only a mention for Portugal in Torrend (1909). Neubert et al. (1995) cite *D. sauteri* for Spain although they do not refer to a collection. Although they give the location as "Extremadura" (Spain), this is probably just a reference to Torrend's record for "Estremadura" (Portugal).

Stemonitopsis microspora (Lister) Nann.-Bremek., Nederlandse Myxomyceten: 208. 1975 ["1974"] PLATE 1·3–4

SPOROCARPS in small groups, stipitate, 1.8–3.5 mm total height. SPOROTHECA cylindrical, subcylindrical or slightly conical with rounded apex, brown, 1.5–2.3 mm long. HYPOTHALLUS shared, brown to black-brown. PERIDIUM evanescent. STIPE 0.6–1.2 mm long, black, with a broadened base. COLUMELLA reaching the apex of the sporotheca, concolorous with the stalk. CAPILLITIUM tortuous,

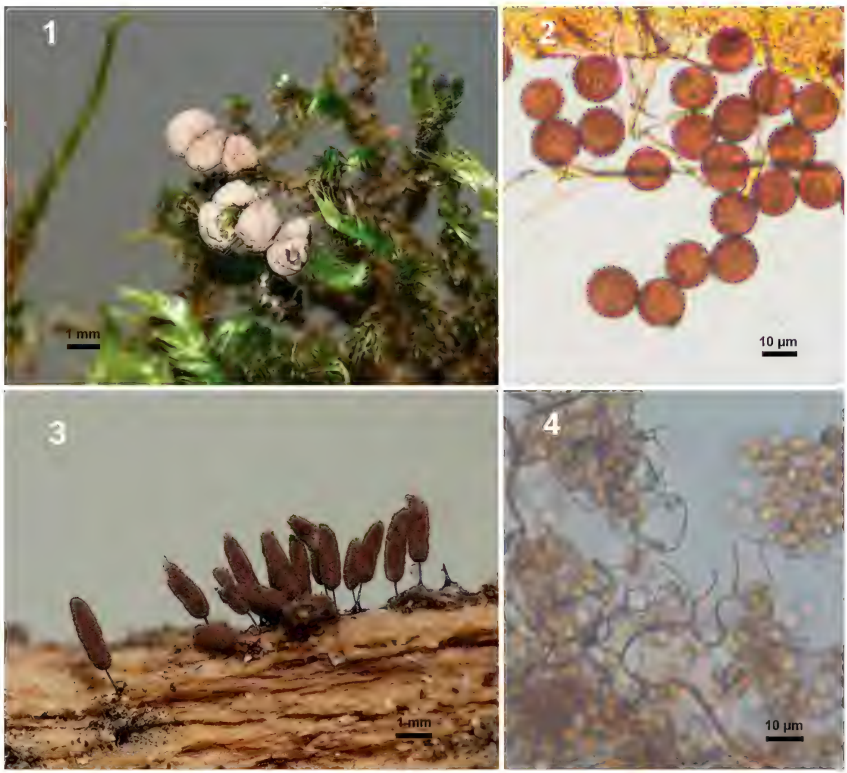

Plate 1. ***Diderma sauteri*** (MA-Fungi 86674). 1. Sporocarps; 2. Echinulate spores. ***Stemonitopsis microspora*** (req-Fungi 500): 3. Sporocarps; 4. Spores and capillitium.

becoming very entangled towards the outer part, branched and anastomosed, forming a fragmented peripheral net. Spores brown to reddish-brown in mass; hyaline, pale brown by LM, subglobose or slightly polygonal, (n = 59) (3.7–)3.9–5.1(–5.2) µm diam, reticulate.

Specimens examined — **SPAIN**: **Pontevedra**. Salceda de Caselas, A Picoña, Río Caselas, 29TNG3676, 66 m, on wood of *Pinus pinaster,* 4-III-2013, leg. Óscar Requejo (req-Fungi 500; MA-Fungi 86675).

Comments — The specimen fructified on *Pinus* wood, although *S. microspora* is often recorded in the literature as growing on leaves (Martin & Alexopoulos 1969; Poulain et al. 2011). All the morphological characters fit well with the description provided by Nannenga-Bremekamp (1975) for the species. The small spore size of *S. microspora* distinguishes it from other species such as *Stemonitopsis hyperopta* (Meyl.) Nann.-Bremek. (5–7 µm diam) and *Comatricha pulchella* (C. Bab.) Rostaf. (6.5–9 µm diam) (Martin & Alexopoulos 1969; Poulain et al. 2011).

According to the data provided by GBIF (2013) there is only one Iberian record of *S. microspora*, in Madrid from 1975. We have found no other bibliographic records of this species for the Iberian Peninsula. The other species of *Stemonitopsis* reported from the Iberian Peninsula are *S. aequalis* (Peck) Y. Yamam., *S. amoena* (Nann.-Bremek.) Nann.-Bremek., *S. hyperopta*, and *S. typhina* (F.H. Wigg.) Nann.-Bremek. (Lado 1993; Hernández-Crespo 2006).

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Four lecideoid lichens new to China

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Abstract — Four lecideoid lichen species — *Bryobilimbia ahlesii*, *B. sanguineoatra*, *Lecidea promixta*, *Porpidia grisea* — are reported for the first time from China.

Key words — *Lecideaceae*, Asia, taxonomy

Introduction

Lecidea (*Lecideaceae*) was originally described by Acharius (1803). In the sense of Zahlbruckner, the genus once represented one of the largest lichen genera and included about 1200 species (Schmull et al. 2011). Subsequently, *Bryobilimbia*, *Porpidia*, and many other obviously more natural units have been excluded based on the molecular data and the structure of the ascomata, especially the nature of the hamathecial tissues, ascus apical structures, and ascospores (Hertel 1975, 1977; Thomson 1997; Buschbom & Mueller 2004; Smith et al. 2009; Fryday et al. 2014). *Lecidea* s. str. is now a medium-sized genus characterized by a *Lecidea*-type ascus (Hertel 2006, Smith et al. 2009). *Bryobilimbia* is distinguished by a hymenium usually with scattered blue-violet (K+ aeruginose-green) granules, a *Porpidia*-type ascus, and ascospores with a warted perispore or thin gelatinous coat (Fryday et al. 2014). *Porpidia* is characterized by strongly branched and anastomosed paraphyses, a *Porpidia*-type ascus, and large halonate ascospores (Gowan 1989, Nash et al. 2004, Smith et al. 2009).

Bryobilimbia includes six species, *Lecidea* s. str. includes about 100 species (Kirk et al. 2008), and *Porpidia* includes about 50 species (Smith et al. 2009; Osyczka & Olech 2011; Fryday et al. 2014; Fryday & Hertel 2014). In China, 16 *Lecidea* s. str. and 16 *Porpidia* species have been reported (Wei 1991; Abbas & Wu 1998; Aptroot & Seaward 1999; Aptroot 2002; Aptroot & Sparrius

2003; Obermayer 2004; Guo 2005; Zhang et al. 2010, 2012; Wang et al. 2012), but no species of *Bryobilimbia* has yet been reported. During our study of lecideoid lichens from China, we identified four species new to the country — *Bryobilimbia ahlesii, B. sanguineoatra, Lecidea promixta*, and *Porpidia grisea*.

Materials & methods

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University) and KUN (Kunming Institute of Botany, Chinese Academy of Sciences). The morphological and anatomical characters of the specimens were examined under a stereo-microscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41). Thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), I (a 10% aqueous solution of aqueous potassium iodide), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol) for identification. The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C (Orange et al. 2010). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Bryobilimbia ahlesii (Hepp) Fryday, Printzen & S. Ekman,
Lichenologist 46(1): 29 (2014) Fig. 1

Morphology — Thallus effuse, thin, irregularly rimose, grey-green; medulla I–; prothallus lacking. Apothecia sessile, 0.45–0.6 mm diam.; disc reddish brown to brown-black, smooth to slightly convex; true exciple usually prominent and persistent, the outer edge hyaline to pale brown, inner exciple and hypothecium brown to blackish brown; epihymenium hyaline to yellowish brown, K–; hymenium hyaline, 60–80 µm tall; subhymenium hyaline to pale yellowish brown, paraphysis c. 1.4 µm wide, apices slightly widened to 2.8–5 µm, simple or branched in upper part, slightly anastomosed, without apical cap or hood. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 12–16(–17.5) × (4–)5–6.25 µm, with or without thin gelatinous coat. Pycnidia not observed.

Chemistry — Thallus and medulla K–, C–, KC–, P–. Hymenium and hypothecium usually with blue-violet (K+ aeruginose-green) granules. No lichen substances detected by TLC.

Distribution — *Bryobilimbia ahlesii* has been reported from Europe and North America (Smith 2009, Halda et al. 2011, Fryday et al. 2014). New to China.

Specimen examined: **CHINA. Heilongjiang**, Wuchang, Mt. Datudingzi, alt. 1150 m, on rock, 21 Aug. 2011, D.F. Jiang 20122253 (SDNU).

Comments — *Bryobilimbia ahlesii* is closely related to *B. sanguineoatra* but differs in wider ascospores and occurring on rock rather than bryophytes.

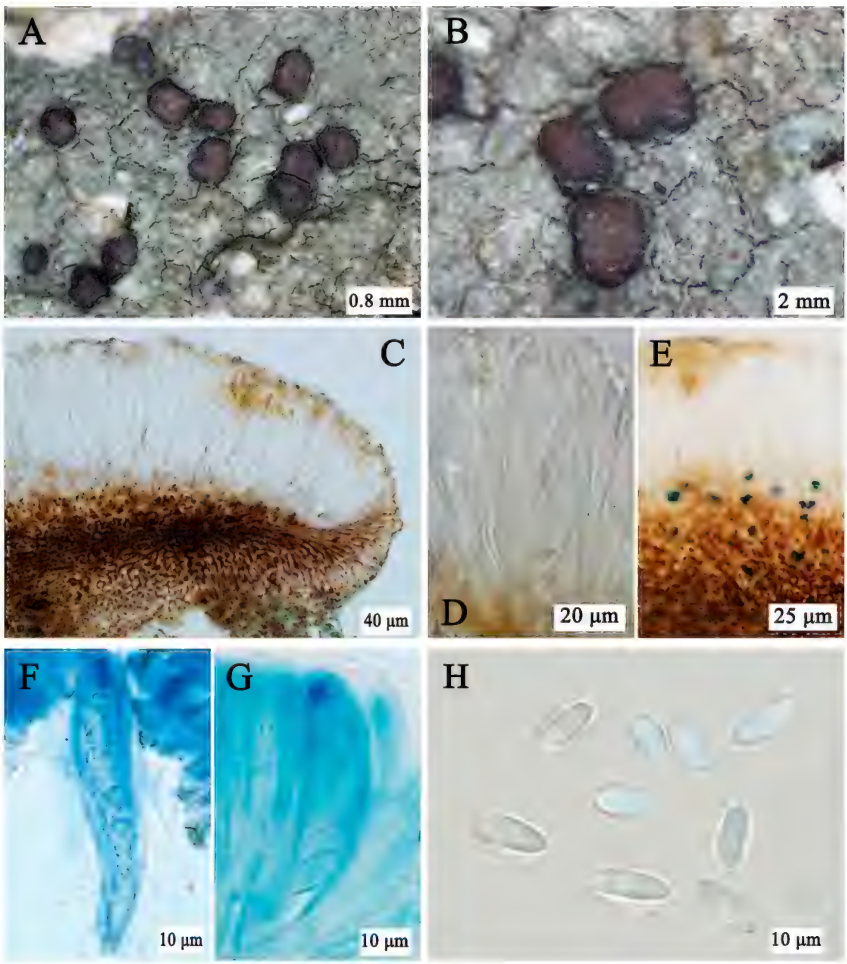

Fig. 1. *Bryobilimbia ahlesii* (Jiang 20122253, SDNU). A: Thallus; B: Apothecia; C: Apothecium section; D: Paraphyses; E: Aeruginose-green K reaction of granules in hymenium and hypothecium; F: Amyloid reaction of ascus; G: Ascus and ascospores; H: Ascospores.

Bryobilimbia sanguineoatra (Wulfen) Fryday, Printzen & S. Ekman, Lichenologist 46(1): 31 (2014) Fig. 2

Morphology — Thallus membranous, thin, pale yellow; I–. Apothecia sessile, 0.7–1.1 mm diam.; disc reddish brown, flat and marginate when young but soon convex and immarginate. True exciple usually exclude, the outer edge pale brown, inner exciple dark reddish brown; epihymenium yellowish brown or almost hyaline; hymenium hyaline, 60–85 µm tall; hypothecium

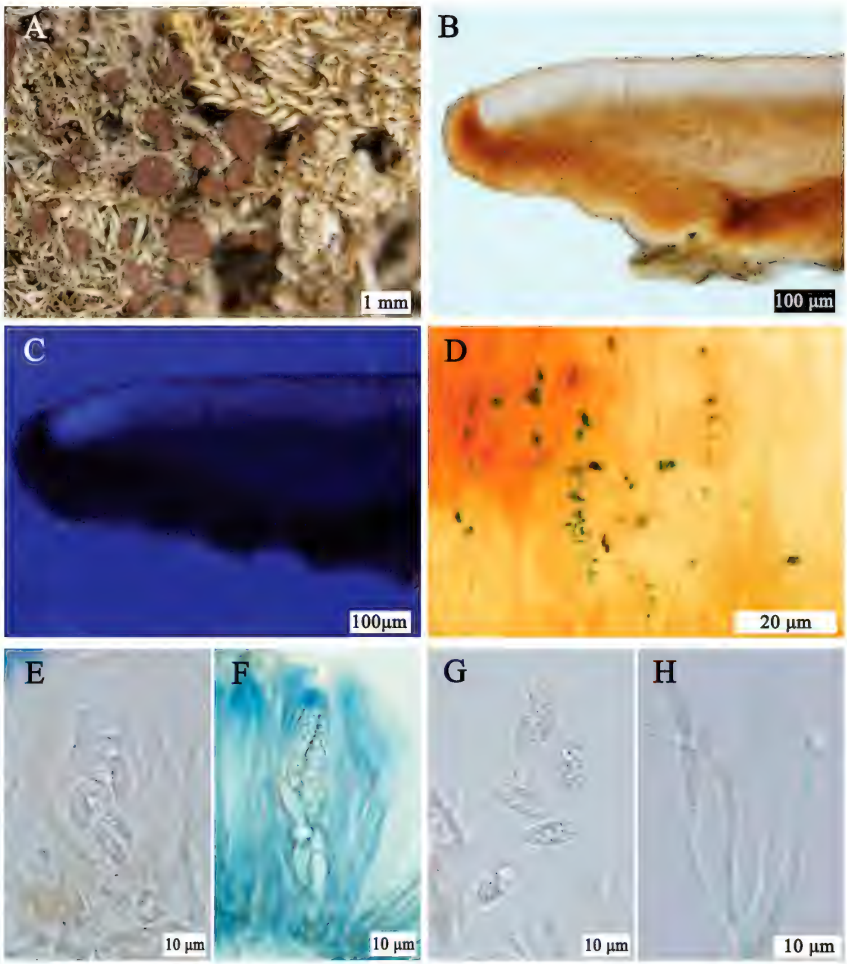

Fig. 2. *Bryobilimbia sanguineoatra* (Wang 14595, KUN). A: Thallus; B: Apothecium section; C: Exciple and epihymenium without crystals; D: Aeruginose-green K reaction of granules in hymenium; E: Ascus and ascospores; F: Amyloid reaction of ascus; G: Ascospores; H: Paraphyses.

above yellowish brown, below usually paler; paraphysis 1.3–1.6 µm wide, apices slightly widened to 2.5 µm, hyaline, mostly simple. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, (9–)11–14 × 3.5–5(–6) µm, perispore not clearly discernable. Pycnidia not observed.

Chemistry — Thallus and medulla K–, C–, KC–, P–. Hymenium and hypothecium usually with blue-violet (K+ aeruginose-green) granules. No lichen substances detected by TLC.

Distribution — *Bryobilimbia sanguineoatra* has been reported from Europe, Macaronesia, Asia, Africa, and North America (Smith et al. 2009, Davydov & Printzen 2012, Fryday et al. 2014). New to China.

Specimen examined: **CHINA. Yunnan**, Zhongdian, Mt. Bitahai, alt. 3400 m, on moss, 21 Sept. 1994, L.S. Wang 14595 (KUN).

Comments — *Bryobilimbia sanguineoatra* is closely related to *B. hypnorum* but differs in having a more readily excluded true exciple and narrower ascospores that are smooth and never septate; in *B. hypnorum*, the ascospores are often

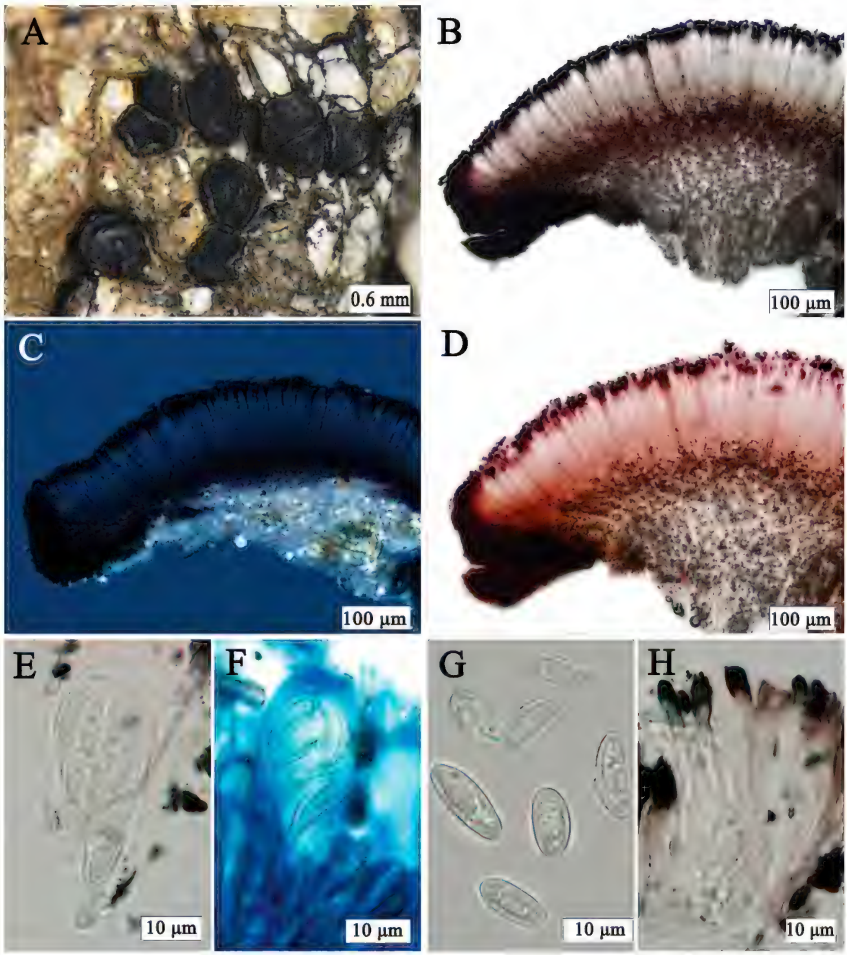

Fig. 3. *Lecidea promixta* (Guo 20127072c, SDNU). A: Apothecia; B: Apothecium section; C: Exciple without crystals; D: Reddish brown N reaction of epihymenium and exciple; E: Ascus and ascospores; F: Amyloid reaction of ascus; G: Ascospores; H: Paraphyses.

thinly 1(–3)-septate, with finely warted perispore, 10–16(–19) × 4.5–6(–7) µm. *Bryobilimbia hypnorum* is distributed in Europe, Macaronesia, Africa, Asia, North America, and the subantarctic islands but is still unknown in China (Smith et al. 2009, Fryday et al. 2014).

Lecidea promixta Nyl., Abh. naturw. Ver. Bremen 14: 490 (1898) Fig. 3

Morphology — Thallus lacking or very indistinct, dark grey, not continuous; hypothallus black; medulla I–. Apothecia sessile, constrict below when mature, 0.3–0.55(–0.65) mm diam.; disc black, plane to convex. True exciple persistent, raised and somewhat swollen, of ± radially arranged swollen hyphae, the outer edge dark brown to black, reddish brown within, without crystals; epihymenium dark green with somewhat brown, irregularly thick, 12–17 mm tall; hymenium hyaline, 40–55 µm tall, I–; hypothecium blackish brown; paraphyses simple or mainly branched only towards the apex, 2.0–3.5 µm wide, the apices capitate and widened to 5.0 µm wide. Asci clavate, *Lecidea*-type; ascospores hyaline, simple, ellipsoid, 10–12.5(–14.5) × 4–5 µm. Pycnidia not observed.

Chemistry — Thallus and medulla K–, C–, KC–, P–. Epihymenium and exciple N+ reddish brown, K–, C–. No lichen substances detected by TLC.

Distribution — *Lecidea promixta* has been reported from Europe (Hertel 2006, Ertz 2008, Liška et al. 2008). New to China.

Specimen examined: **CHINA. Shaanxi**, Baoji, Mt. Taibaishan, alt. 3750 m, on rock, 5 Aug. 2005, S.X. Guo 20127072c (SDNU).

Comments — *Lecidea promixta* is closely related to *L. promiscens*, which also has a dark green or green-brown epihymenium and dark brown hypothecium. However, *L. promiscens* contains confluentic acid syndrome, and the medulla is I+ deeply violet (Smith 2009, Zhang et al. 2012), and in China is distributed in Yunnan (Wang 00-19783; KUN).

Porpidia grisea Gowan, Bryologist 92(1): 48 (1989) Fig. 4

Morphology — Thallus crustose, subrimose to rimose-areolate, even to weakly verruculose or rugulose, 0.2–0.7 mm thick, medium gray to pale greenish gray; medulla I+ violet-black; prothallus continuous between thallus patches, black, thin; soredia absent. Apothecia scattered, soon becoming sessile, 0.7–1.8(–2.2) mm diam.; disc black, plane to weakly convex, usually moderately pruinose; pruina grayish white; margin bare, distinct, even to weakly crenulate. Exciple greenish to brownish black at exciple margin, dark brown within, 87.5–140 µm wide, without crystals, with parallel-radiate hyphae, hyphae c. 4 µm wide; epihymenium brown or brownish yellow, without crystals; hymenium hyaline, 80–100(–112.5) µm tall, I+ blue; subhymenium 25–37.5 µm, hypothecium blackish brown; paraphyses strongly anastomosed

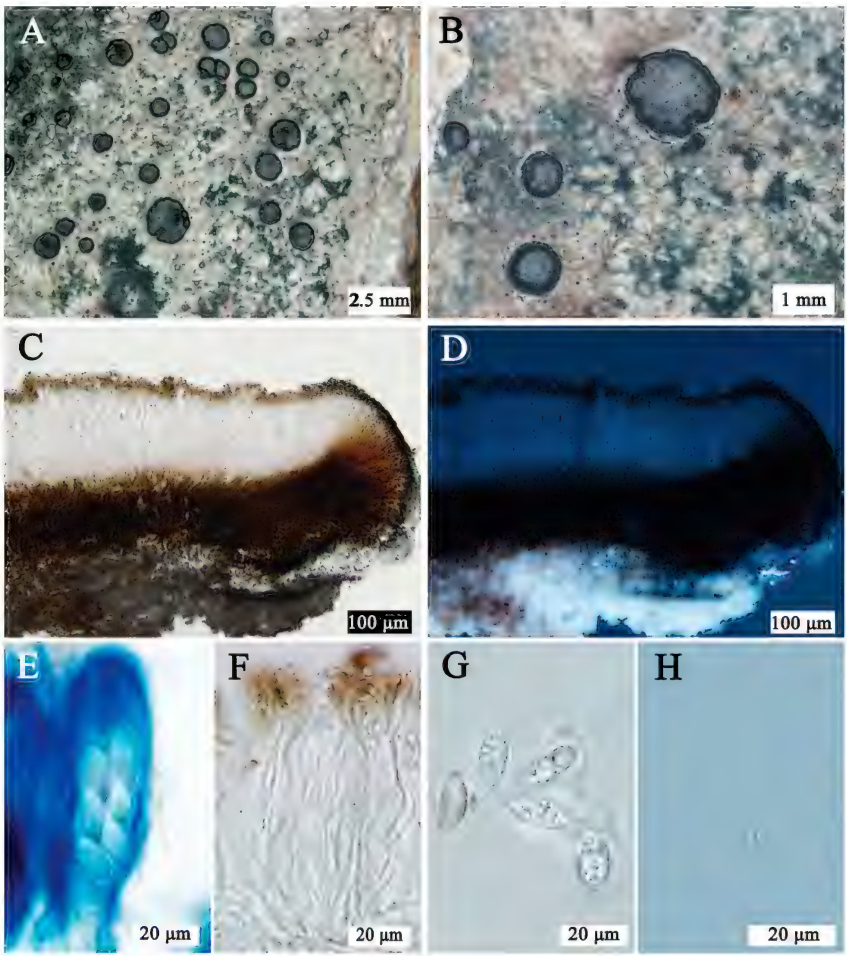

Fig. 4. *Porpidia grisea* (Zhang 20100626, SDNU). A: Thallus; B: Apothecia; C: Apothecium section; D: Exciple and epihymenium without crystals; E: Amyloid reaction of ascus; F: Paraphyses; G: Ascospores; H: Conidia.

and apically branched. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 15–20(–25) × 7–8.5(–10) µm, halonate. Pycnidia immersed, conidia bacilliform, 7.5–11.5 × c. 1 µm.

Chemistry — Thallus and medulla K–, C–, KC–, P–. Confluentic acid, 2'-O-methylmicrophillinic acid and 2'-O-methylperlatolic acid were detected by TLC.

Distribution — *Porpidia grisea* has been reported from Europe and North America (Nash et al. 2004). New to China.

Specimen examined: CHINA. Xizang, Ridong, Mt. Qimala, alt. 4600 m, on rock, 26 Sep. 1982, M. Zang 4540 (KUN). Yunnan, Dali, Mt. Cangshan, alt. 3955 m, on rock, 31 Jul. 2013, L.L. Zhang YN0116, YN0121, YN0135 (SDNU); Deqin, Mt. Baimaxueshan, alt. 4760 m, on rock, 5 Oct. 2009, L.S. Wang 09–31110 (KUN)); Lijiang, Mt. Laojunshan, alt. 3800 m, on rock, 5 Nov. 2009, H.Y. Wang 20100624, 20100625, 20127130, L.L. Zhang 20100626 (SDNU).

Comments — *Porpidia grisea* grows on siliceous or calcareous rock, it is closely related to *P. tuberculosa* and *P. speirea*, all of which belong to the *Porpidia speirea* complex, which produces moderately pruinose apothecia, I+ medulla, and confluentic acid. However, *P. tuberculosa* always has soredia, and the apothecia rarely seen; *Porpidia speirea* usually has a chalky white thallus, and apothecia that remain sunken or very broadly sessile when mature; it grows only on calcareous rock and in China is distributed in Xinjiang (Abdulla 920442, 920455a, 920728, 920746; XJU).

Acknowledgements

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Muscodor strobelii, a new endophytic species from South India

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Abstract — *Muscodor strobelii* is described as a new endophytic species existing in stems of *Cinnamomum zeylanicum* from South India based on morphological and molecular evidences along with volatile organic properties.

Key words — antimicrobial activity, sterile ascomycete, cinnamon, ITS- rDNA

Introduction

Plants hold an enormous diversity of fungal and bacterial endophytes within leaf and stem tissues, without any obvious indication of their presence (Bacon & White 2000, Schulze & Boyle 2005, Strobel 2006, Murali et al. 2007, Hyde & Soytong 2008, Rodriguez et al. 2009, Peay et al. 2010). Endophytes have been recognized as a promising resource of bioactive compounds and phytochemicals, which find applications in medicine, industry, and agriculture (Strobel & Daisy 2003, Mitchell et al. 2008, Tejesvi et al. 2009, Aly et al. 2010, Gutierrez et al. 2012).

Eight sterile endophytic fungi emanating distinct musky odours due to presence of volatile organic compounds (VOCs) have been described as novel fungi: *Muscodor albus* from *Cinnamomum* in Honduras (Worapong et al. 2001); *M. cinnamomi* from *Cinnamomum* in Thailand (Suwannarach et al. 2010); *M. crispans* from *Ananas* in Bolivia (Mitchell et al. 2008); *M. fengyangensis* from *Actinidia* and *Pseudotaxus* in China (Zhang et al. 2010); *M. roseus* from *Grevillea* in Australia (Worapong et al. 2002); *M. sutura* from *Prestonia* in Colombia (Kudalkar et al. 2012); *M. vitigenus* from *Paullinia* in Peru (Daisy et al. 2002); and *M. yucatanensis* from *Bursera* in Mexico (Gonzalez et al. 2009).

Apart from morphological and molecular differences, gas chromatography and mass spectroscopy (GC/MS) of VOCs have also been used as a tool to demarcate different species of *Muscodor* (Strobel et al. 2001, Kudalkar et al.

2012). *Muscodor* species exhibit biological control properties due to their VOC admixtures that exhibit antibiotic properties and induce lethal effects on insects and nematodes.

In this study, we recovered an endophytic fungus (#6610) from the stem tissue of a cinnamon tree (*Cinnamomum zeylanicum*) growing in Biligiri Rangaswamy Temple (BRT) wildlife sanctuary, India. Its cultural, chemical, and molecular characters are distinct from previously described *Muscodor* species, and so we name it here as *Muscodor strobelii*.

Materials & methods

Fungal isolation

Healthy and mature twigs were collected from *Cinnamomum zeylanicum* growing in BRT Wildlife Sanctuary, Karnataka, India, during July 2009. The plant parts were placed in sterile zip pouches and stored at 4 °C until further use. Fungi were selectively isolated using VOC stress assay as described by Ezra et al. (2004) using the *Muscodor albus* CZ620 type strain (gifted by Professor Gary A. Strobel, Montana State University, USA). The twigs were cut into 1–2 cm pieces under aseptic conditions, surface sterilized (with 1% sodium hypochlorite for 5 min, 70% ethanol for 1 min, and 30% ethanol for 30 s), and air dried under a laminar hood. The sterilized plant samples were cut into 5–10 mm segments, placed into a potato dextrose agar (PDA) plate containing a 7-day old culture of *M. albus* CZ620, and sealed with parafilm. The plates were incubated at 24 ± 2 °C for a fortnight with a 12 h photoperiod and periodically observed for mycelial germination. The fungi growing out of the plant tissue were aseptically picked from the tip and transferred to fresh PDA plates so as to obtain pure isolates. VOCs produced by *M. albus* CZ620 would permit growth of only volatile-tolerant fungi, thus enhancing the probability of finding a new *Muscodor* isolate or strain. The pure isolates thus obtained were maintained on PDA slants. Combinations of various media [Richard's agar (RA), corn meal agar (CMA), banana leaf agar (BLA), malt extract agar (MEA)] and growth conditions were tested to stimulate spore production (Guo et al. 1998). The morphological characters (e.g., colony color, texture, growth pattern, pigment formation, VOCs) and microscopic structures (e.g., hyphal characteristics, cellular bodies) were observed (Mitchell et al. 2008). The holotype was deposited as a metabolically inactive culture (liquid nitrogen) at National Fungal Culture Collection of India, Agharkar Research Institute, Pune (NFCCI).

Scanning electron microscopy

The morphology of isolate #6610 was observed under the scanning electron microscope following Ezra et al. (2004). The samples were placed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) overnight at 4 °C. The next day these were washed twice with 0.1 M phosphate buffer for 10 minutes each, and then washed for 10 minutes each in 30%, 50%, 60%, 70%, 80%, 90%, 95% and 100% acetone. The samples were then dehydrated to critical point using liquid CO_2. Gold palladium coating of dehydrated fungal samples was done using a sputter coater and images were recorded in high vacuum mode using Zeiss Evo40 scanning electron microscope (Ezra et al. 2004, Kudalkar et al. 2012).

Fungal DNA isolation and amplification

Isolate #6610 was grown on PDA in a 90 mm petri dish for 7 days, after which the mycelium was harvested, freeze dried in liquid nitrogen, and ground into a fine powder with pestle and mortar. About 10 mg of powdered mycelium was transferred to 2 ml microfuge tube and genomic DNA extracted using Wizard® Genomic DNA purification kit (Promega, USA) as per manufacturer's protocol.

The internal transcribed spacer (ITS) regions 1 and 2 and 5.8S rDNA regions were amplified using *Muscodor* specific primers *M. albus* F (5′-GGGAGGCTACCCTATAGGGGATAC-3′) and *M. albus* R (5′-CAGGGGCCGGAACCAC TACAGAGG-3′) (Ezra et al. 2010). The 25 µl reaction mixture for ITS amplification comprised 1 µl of extracted fungal DNA, 10 µM of each primer, 2.5 mM of dNTP, 25 mM $MgCl_2$, and 1.5 U of Taq DNA polymerase in 10 X Taq buffer (Bangalore GeNei). Thermal cycling conditions for *Muscodor* specific primers was initial denaturation at 96 °C for 5 min followed by 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s, followed by final extension at 72 °C for 5 min. The ITS amplicons were examined using a 1.5% agarose gel under UV light in Bio-Rad Gel documentation System using Quantity-1-D analysis software. The PCR products were purified with Wizard® SV gel and PCR clean up system (Promega, USA) following manufacturer's protocol. The purified products were directly sequenced (Chromus Biotech, Bangalore) using PCR primers mentioned above.

Sequence assembly, alignment and phylogenetic analysis

Sequences were assembled using Sequencher ver. 5 (www.genecodes.com) and compared to GenBank sequences using the BLAST software on the NCBI website. The ITS sequences were aligned with selected sequences of reference taxa obtained from BLAST using the Clustal W. Any duplicate sequence types were removed from the data set prior to phylogenetic analyses. After multiple alignments, phylogenetic analysis was conducted in MEGA 5.0.5. A phylogeny comparing *Muscodor* species #6610 with related taxa was generated by the Neighbour-Joining method (Saitou & Nei 1987). The evolutionary distances (base substitutions per site) were computed using the Kimura 2-parameter method (Kimura 1980). The rate variation among sites was modeled with a gamma distribution (shape parameter = 5). Alignment gaps were designated as missing data. Clade stability was assessed by bootstrap analysis with 1000 bootstrap replicates. The phylogenetic tree (Fig. 8) was drawn and edited in MEGA 5.0.5.

Qualitative analysis of volatiles

The volatile organic gas mixture emanated by the 10-day old isolate #6610 culture was entrapped using a solid phase micro-extraction (SPME) syringe with a stable flex fibre of 50/30 di-vinylbenzene/carboxen on polydimethylsiloxane (Supelco, Sigma Aldrich) following Ezra & Strobel (2004). The fibre was exposed to the air space above the fungus for 45 min by placing the SPME syringe after drilling a small hole with the help of sterile needle. The exposed fibre was injected for 30 s in the Shimadzu QP 2010 + gas chromatograph with thermal desorption system TD 20. A RTX column (diphenyl 95%, dimethyl polysiloxane 5%) with 30 m × 0.25 mm ID and 0.25 mm DF was used to separate the fungal volatiles. The column was programmed at 100 °C for 2 minutes before the temperature was increased to 250 °C for 2 minutes and finally to 300 °C

for 13 minutes. The carrier gas was helium and the initial column head pressure was 94.4 KPa. Data acquisition and processing was done on GCMS solution software. The compounds obtained after GC/MS analysis was then subtracted from the control plate consisting only PDA medium. The obtained compounds were then tentatively identified based on their high quality matching with database of National Institute of Standard and Technology (NIST) compounds (NIST05) and compared with all reported species of *Muscodor* (Ezra et al. 2004, Kudalkar et al. 2012).

Bioassay of VOCs produced by *Muscodor strobelii*

Antimicrobial spectrum of volatiles produced by *Muscodor strobelii* (#6610) was tested by uncomplicated bioassay using 90 mm four-quadrant petri dishes (Ezra et.al. 2004). A divided petri dish was used to prevent movement of any diffusible inhibitory compound from *M. strobelii* to the test panel microorganisms through the medium. One quadrant of the petri plate was inoculated with the mycelial plug of an actively growing *M. strobelii* culture and the plates were sealed with parafilm and incubated for 5 days at 24 ± 2 °C for production of the VOCs. Subsequently test bacteria and yeast were streaked in rest of the quadrants. While testing filamentous pathogenic fungi 3 mm mycelial plugs were inoculated. Correspondingly the control plates comprised only inoculated test bacteria or fungi and were devoid of isolate #6610 allowing it to grow normally. The antimicrobial action of VOC was determined by monitoring the growth of the test organism in the control and test plates. To check the VOC inhibitory or killing effect after 3 days exposure, the exposed culture plugs were placed on fresh solid media (PDA, YEPD, MHA) to assess their viability (Mitchell et al. 2010).

Taxonomy

Muscodor strobelii Meshram, S. Saxena & N. Kapoor, **sp. nov.** Figs 1–7

MycoBank MB802337

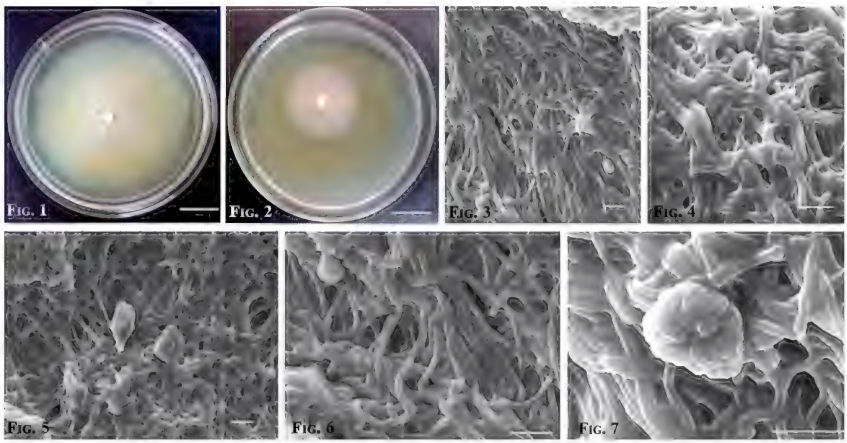
Differs from *Muscodor roseus* by its whitish to pale yellowish-orange mycelium and the absence of hyphal coils.

Type: India, Karnataka, BRT Wildlife Sanctuary, 11°43′–12°09′N 77°01–15′E as endophytic fungi from internal tissue of stem of *Cinnamomum zeylanicum* Blume (*Lauraceae*), 12 July 2009, leg. Sanjai Saxena #6610 (**Holotype,** NFCCI-2907; Gen Bank, JQ409999).

Etymology: The epithet *strobelii* honors Dr. Gary Strobel, Emeritus Professor, Department of Plant Sciences, Montana State University, Bozeman MT, USA.

Teleomorph: Unknown

In nature, the fungus is associated with *Cinnamomum zeylanicum* and is an ascomycete with sterile mycelia. The fungal colonies grew slowly over PDA and MEA media, with colony diameter of 17–18 mm on PDA and 27–29 mm on MEA when incubated at 24 ± 2 °C for 7 days with 12 hours of photoperiod. Fungal colonies on PDA are initially floccose and whitish but with age turn pale yellowish-orange. Over PDA it generates hyaline, septate branched hyphae that are 0.76–1.5 µm thick. Hyphae form fused cables that interweave into fishnet-like structures, which further merge to form zinnia flower- or bud-like



Figs. 1–7. *Muscodor strobelii* (#6610). 1. 30-day old colony on PDA. 2. Ropy appearance of mycelium after 6-day incubation on malt extract agar. 3. Mycelial arrangement of 10-day old culture on PDA (SEM). 4.Fishnet-like structures (SEM). 5–6. Zinnia flower- and bud-like structures after 10 days on PDA (SEM). 7. Enlarged view of zinnia flower-like structure (SEM). Bars: 1–2 = 2 cm; 3–7 = 10 μm.

structures (5.5–15.8 μm). The hyphal fabrication is denser in MEA, where the isolate produces interwoven mycelia that form a 0.76–2.04 μm diam. rope-like structure. The isolate did not produce any fruiting or conidial structure under tested in vitro conditions.

This *M. strobelii* isolate was found viable against the VOCs produced by *M. albus* CZ620 in the VOC stress bioassay on repeated exposure.

Detailed morphological studies using scanning electron microscopy

The mycelium of *M. strobelii* in SEM studies exhibits typical characters of a *Muscodor* species in forming rope-like strands that branch at a specific angle (Fig. 3). The cable-like mycelium further forms an interwoven ropy structure much like fishing net (Fig. 4) that culminates to form a unique structures at its terminal end to resemble a common zinnia flower- (as seen from the top) or bud-like (as seen from the side) structure (Figs. 5–7).

As these structures do not germinate further or sporulate, they should not be considered to be fruiting bodies. Morphologically, *M. strobelii* differs from *M. crispans* and *M. cinnamomi*, which exhibit cauliflower-like sterile structures and possess ropy coiled mycelia. *Muscodor yucatanensis* has a ropy structure with swollen hyphae while *M. albus* exhibits only a ropy mycelium, but both lack the sterile structures produced by *M. strobelii*. *Muscodor roseus,* with its dense rose coloured mycelium with a variable hyphal thickness that forms unique hyphal coiled structures, is remarkably dissimilar from *M. strobelii*.

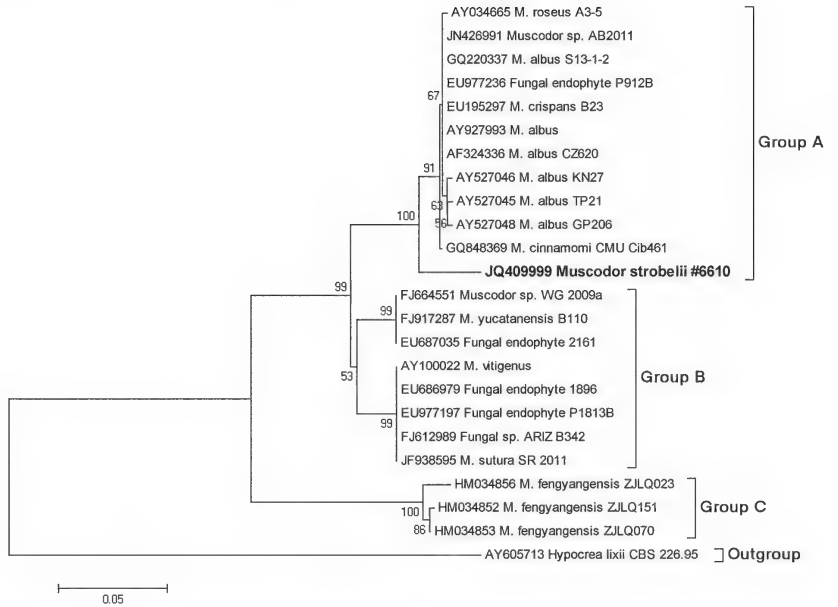


FIG. 8. Phylogeny of *Muscodor* spp.: Neighbor Joining tree based on ITS rDNA region. The optimal tree with the sum of branch length = 0.59173282 is shown. There were a total of 632 positions in the final dataset.

Molecular phylogeny of *M. strobelii*

The advanced BLAST search of the partial ITS1-5.8S-ITS2 rDNA region of *Muscodor strobelii,* which revealed a 94% sequence similarity with *M. cinnamomi* (GQ848369; Suwannarach et al. 2010), *M. crispans* B23 (EU195297; Mitchell et al. 2008), *M. albus* CZ620 (Worapong et al. 2001), *M. albus* E6, and *M. albus* GP206 and a 92% sequence similarity with *M. yucatanensis*, indicates that the isolate belongs to *Muscodor* genus (TABLE 1).

Representative ITS accessions of *Muscodor* species and unknown fungal endophytes (TABLE 1) were analyzed to establish possible phylogenetic relationships with *M. strobelii* through an alignment matrix using Clustal W, MEGA 5.0.5 analysis. The resultant phylogenetic tree divided into 3 major groups (Group A, B and Group C). Group A clustered *M. crispans, M. roseus*, *M. cinnamomi* CMU-Cib46, *M. albus* CZ620, 6 strains of *M. albus*, and one unknown fungal endophyte 912B with 91% bootstrap support; our isolate was found to be basal to group A with 100% bootstrap support. Group B comprised *M. yucatanensis, M. vitigenus, M. sutura, Muscodor* sp. WG2009a, and 4 unknown fungal endophytes. *Muscodor fengyangensis* species forms a separate group (Group C) with 100% bootstrap support; *Hypocrea lixii* CBS 226.95 was

Table 1. Advanced ITS-5.8 rDNA gene BLAST search homology analysis of *Muscodor strobelii.*

Species / voucher	GenBank no.	Query coverage	Sequence similarity
M. cinnamomi CMU Cib-461	GQ848369	98%	94%
M. crispans B23	EU195297	98%	94%
M. albus CZ620	AF324336	98%	94%
M. roseus A3-5	AY034665	98%	94%
M. sutura CA22-D	JF938595	91%	91%
M. vitigenus	AY100022	91%	91%
M. yucatanensis	FJ917287	90%	92%
M. fengyangensis ZJLQ070	HM034853	90%	90%
M. albus S13-1-2	GQ220337	98%	94%
M. albus I-41.3s	AY927993	98%	94%
M. albus TP21	AY527045	98%	94%
M. albus KN27	AY527046	98%	94%
M. albus GP206	AY527048	98%	94%
Muscodor sp. WG2009a	FJ664561	90%	92%
Fungal endophyte 912B	EU977236	98%	94%
Fungal endophyte 2161	EU687035	90%	92%
Fungal endophyte 1896	EU686979	91%	100%
Fungal endophyte P1813B	EU977197	91%	91%
Fungal sp. ARIZ B342	FJ612989	91%	91%

included as outgroup (Fig. 8). These data delineate *Muscodor strobelii* from the already described *Muscodor* species and highlight its novelty.

Bioassay of *Muscodor* species VOCs against human and plant pathogens

The volatiles emanated by *M. strobelii* #6610 exhibited a broad spectrum of activity against yeasts, bacteria, and filamentous fungi. Complete inhibition was observed in clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* after three days of exposure. The VOCs were bactericidal, as the test bacteria had lost their viability when they were re-inoculated on fresh medium. The test *Candida* isolates also showed complete inhibition when exposed to the *M. strobelii* VOC mixture and lost their viability.

Among the filamentous fungi, only *Penicillium citreonigrum*, *Botrytis cinerea*, and *Aspergillus japonicus* were completely inhibited by the VOC followed by *Cercospora beticola* and *Mycosphaerella fijiensis,* inhibited by 60–70%. The completely inhibited fungi were non-viable, whereas *Cercospora beticola*, *Rhizoctonia solani,* and *Colletotrichum gloeosporioides* showed delayed

Table 2. Antimicrobial activity of volatile organic compounds (VOCs) produced by *Muscodor strobelii* after 3 days exposure.

Test Cultures	Repository	% Growth (3 days exposure)
Bacillus subtilis	MTCC 441	70.3 ± 1.5
Escherichia coli	GMCP	0
Pseudomonas aeruginosa	GMCP	0
Staphylococcus aureus	GMCP	0
Candida albicans	JNU	0
Candida albicans	MTCC 3019	0
Candida albicans	MTCC 183	0
Penicillium citreonigrum	MTCC 160	0
Botrytis cinerea	MTCC 359	0
Aspergillus japonicus	MTCC 1975	0
Mycosphaerella fijiensis	MSU	43 ± 2.64
Cercospora beticola	MSU	32 ± 2
Rhizoctonia solani	MTCC 4634	60.7 ± 1.15
Colletotrichum gloeosporioides	MTCC 9623	63 ± 2
Fusarium oxysporum	DBTES, TU	70 ± 4
Lasiodiplodia theobromae	DBTES, TU	60 ± 4

*The growth pattern was compared with that of the control plate where the test bacteria, yeast, and filamentous fungi grew under no stress conditions. All tests were performed in triplicate; their mean ± SD was calculated.

Repositories:
DBTES, TU: Department of Biotechnology and Environmental Sciences, Thapar University, Patiala.
JNU: Jawaharlal Nehru University, New Delhi.
GMCP: Government Medical College, Patiala.
MSU: Montana State University, USA.

sporulation compared to the control (Table 2). The *M. strobelii* VOCs are active on fungi as well as bacteria. *Muscodor albus, M. crispans,* and *M. fengyangensis* also exhibit antibacterial as well as antifungal activity; however differential activity exists among the tested bacterial and fungal isolates, which might be attributed to the chemistry and quantity of the volatiles being produced.

Volatile compounds of *Muscodor strobelii*

A 10-day old culture of *M. strobelii* #6610 produced at least 14 volatile moieties that could be possibly identified based on GC/MS comparison of authentic standards obtained from commercial sources as well as by organic synthesis. Primarily the compounds were identified based on their mass spectral properties when compared to the NIST database. Of all the compounds produced, 4-octadecylmorpholine was the most abundant, with the highest peak area (12.5%). The other unique volatile compounds produced were Tetraoxapropellan, Aspidofractinine-3-methanol, 1-(3,5-di-tert-butyl-

Table 3. Volatile compounds[1] produced by *Muscodor strobelii*.

Retention time		Possible name	Molecular Formula	Mass (Da)
3.03	1.76	1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	$C_{10}H_{16}$	136.23
3.69	4.56	Terpinolene	$C_{10}H_{16}$	136.23
13.13	3.5	2-morpholinoethanamine	$C_6H_{14}N_2O$	130.18
15.06	8.57	1-(3,5-di-tert-butyl-4-hydroxyphenyl)propan-1-on	$C_{17}H_{26}O_2$	262.38
15.33	9.35	Tetraoxapropellan	$C_{10}H_{16}O_4$	200.24
15.92	8.07	Viridiflorol	$C_{15}H_{26}O$	222
16.30	4.11	3,5-di-tert-butylcyclohexa-3,5-diene-1,2-dione	$C_{14}H_{20}O_2$	220.30
16.94	8.04	2-(6-tert-butyl-1,1-dimethyl-2,3-dihydro-1H-inden-4-yl)acetic acid	$C_{17}H_{24}O_2$	260
17.22	2.96	4-(2-morpholinoethyl)-4H-1,2,4-triazole-3-carboxamide	$C_9H_{15}N_5O_2$	225.25
20.193	1.08	1,2,3,4-tetramethyl-5,6-dineopentylbenzene	$C_{20}H_{34}$	274.48
21.178	12.53	4-octadecylmorpholine	$C_{22}H_{45}NO$	339.6
24.798	3.62	octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5
32.054	9.23	Aspidofractinine-3-methanol	$C_{20}H_{26}N_2O$	310.4
32.385	2.15	(2E)-2-(3-methoxy-5-methyl benzylidene)-7-methyl-1-indanone	$C_{19}H_{18}O_2$	278

[1] After 10 days incubation at 24 ± 2 °C on potato dextrose agar (PDA) using solid-phase micro-extraction (SPME) fibre and GC/MS analysis

4-hydroxyphenyl)propan-1-one, viridiflorol, terpinolene, 2-(6-tert-butyl-1,1-dimethyl-2,3-dihydro-1H-inden-4-yl)acetic acid, 3,5-di-tert-butylcyclohexa-3,5-diene-1,2-dione, and 2-morpholinoethanamine (Table 3). The volatiles commonly produced by other *Muscodor* species consist of propanoic acid, 2-methylesters, azulene and naphthalene derivatives, and thujopsene. *Muscodor strobelii* produces volatiles belonging to ketones, amines, carboxylic acids, and alcohols; they are unique and have not been reported from any other *Muscodor* species.

Conclusions

As *Muscodor* species are sterile and do not produce any reproductive structures, critical examination of cultural, physiological, and biochemical properties along with molecular phylogenetic data is essential for verifying them as distinct species. *Muscodor strobelii* exhibited a variety of common features shared by *Muscodor* species; it possessed, however, some unique features like differences at the molecular level, unique morphological features seen through scanning electron microscopy, differing antimicrobial susceptibility data, and a unique volatile gas mixture composition (Table 4). Thus *M. strobelii* is introduced as a novel species that possesses potential as a mycofumigant.

TABLE 4. Comparison of *M. strobelii* with other *Muscodor* species.

	STROBELII	*ALBUS**	*CINNAMOMI**	*CRISPANS**	*FENGYANGENSIS**	*ROSEUS**	*SUTURA**	*VITIGENUS**	*YUCATANENSIS**
MYCELIAL GROWTH	Rope-like, slimy; Zinnia- & bud-like bodies	Rope-like	Rope-like with coiled hyphae	Rope-like with cauli-flower-like bodies	Rope-like with coiled hyphae	Rope-like, forming erumpent pie-shaped sectors	Rope-like bands extra-cellular bodies	Rope-like	Rope-like with coiled hyphae
HYPHAL GROWTH (at colony front)	Straight	Straight	Straight	Heavily wavy new growth	Straight	Intertwining, rope-like, (occasionally coiled)	Suture-like pattern on PDA (new growth)	Straight	Multiple rope-like strands (can inter-twine to form coils)
PIGMENT PRODUCTION	Pale yellow (in light)	None	Pale orange (in light)	Reddish (in light)	Yellow	Light rose	Reddish (in dark)	None	None
MAJOR VOCs	4-octadecyl-morpholine, Tetraoxa-propellan, Aspido-fractinine-3-methanol	Propanoic acid, 2-methyl, 2-nonanone, Naphthalene & azulene derivatives	Propanoic acid, 2-methyl, Methyl ester, β-humulene	Propanoic acid, 2-methyl, (+ many esters)	Naphthalene & azulene derivatives (+ many others)	2-butenoic acid, Ethyl ester, 1,2,4-tri-methyl-benzene, 2-nonadiene	Propanoic acid, 2-methyl, Thujopsene	Naphthalene (only)	1R,4S,7S, 11R-2,2,4,8-Tetramethyl-tricyclo [5.3.1.0(4,11)] undec-8-ene, Caryophyl-lene, Aroma-dendrene
BIOACTIVITY									
Antifungal	+	+	+	+	+	+	+	–	+
Antibacterial	+	+	+	+	+	+	–	–	–
Anti-insect	–	–	–	–	–	–	–	+	–

* Data collated from the protologue publications

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New record of *Setomelanomma holmii* on *Picea crassifolia* in China based on morphological and molecular data

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Abstract — *Setomelanomma holmii* (*Phaeosphaeriaceae*) was collected for the first time in China and for the first time on *Picea crassifolia*. This represents a new generic record for Chinese mycobiota. The fungus is identified based on morphological and molecular characters. An illustrated description of the species is provided.

Key words — ITS, SNEED, SSU, taxonomy

Introduction

During an investigation of fungal diversity of *Picea crassifolia* Kom. in different areas of Kangle County, Gansu Province, China, several samples were recovered with the morphological characteristics of *Setomelanomma holmii*. A literature survey indicated that the genus had not been previously reported from China (Tai 1979, Qiao et al. 2011, Zhang et al. 2012, Xu et al. 2013).

Setomelanomma M. Morelet is a monotypic genus typified by *S. holmii*, which was initially reported in France (Morelet 1980). Later, morphological studies and phylogenetic analysis inferred from SSU nrRNA regions indicated that *S. holmii* occurs also in North America (Canada, United States) and possibly South Korea (Rossman et al. 2002, Zhang et al. 2009, Kim et al. 2011, Plewa et al. 2012).

Materials & methods

Morphological studies. — The specimens and pure cultures were deposited at the herbarium of Museum of Beijing Forestry University, Beijing Forestry University (BJFC). Photographs of features were taken using a Nikon P500 digital camera (Nikon, Tokyo).

The microscopic procedures follow Rossman et al. (2002). Microscopic observations and measurements were made from the slide preparations and photographs taken using a Leica 4D07 microscope (Leica, German). Spore sizes are presented as the range ± 5%, and n = number of spores measured. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analyses. — Genomic DNA was extracted from pure cultures grown in potato dextrose agar (PDA) at 25°C for 2 weeks. A Wizard® Genomic DNA Purification Kit (Finnzymes) procedure was used to extract total genomic DNA from the fruitbodies for polymerase chain reaction (PCR). DNA sequencing was performed at Beijing Genomics Institute. The nITS region was amplified with primer pairs ITS1 and ITS4 (White et al. 1990) and the nSSU region with primer pairs NS1 and NS4 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). We submitted our newly generated sequences to GenBank as *Setomelanomma holmii* (ITS, KF668244; nSSU, KF668245–KF668247) and aligned them with additional sequences downloaded from GenBank, using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). The sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/; submission ID: 14727). The SSU nrRNA sequences underwent maximum parsimony analysis using *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not., *Xylaria hypoxylon* (L.) Grev., and *Hypocrea schweinitzii* (Fr.) Sacc. as outgroups (Rossman et al. 2002). The tree was constructed in PAUP* v.4.0b10 (Swofford 2002) with all characters equally weighted and gaps treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics such as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated for each Maximum Parsimonious Tree (MPT) generated. MrMODELTEST v.2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BY). Bayesian inference was calculated with MrBayes v.3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 500,000 generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

Taxonomy

Setomelanomma holmii M. Morelet, Bull. Soc. Sci. Nat. Arch. Toulon Var 36: 15. 1980. Fig. 1

Ascomata solitary, scattered, initially immersed, subcuticular, becoming erumpent, superficial through periderm of small twigs with needles still attached, perithecioid, 105–279 µm diam, black, globose to subglobose, each with a non-rostrate, periphysate ostiolum, with scattered, sparse to abundant

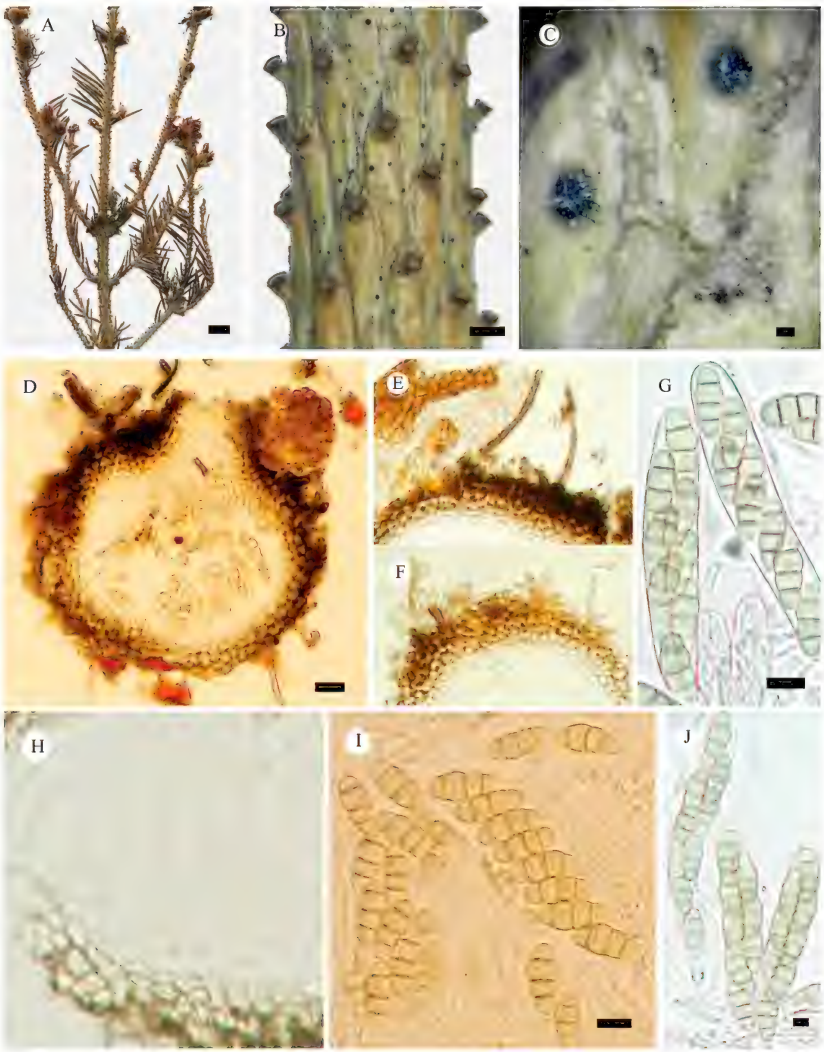

FIGURE 1. *Setomelanomma holmii*. A: Infected twig of *Picea crassifolia* (BJFC-S425). B–C: Ascomata on twig (BJFC-S423). D: Longitudinal section of ascomata (BJFC-S425). E–F: Setae on ascomata (BJFC-S424). G, J: Asci (BJFC-S425). H: Interthecial elements among immature asci (BJFC-S428). I: Ascospores (BJFC-S425). Scale bars: A = 1 cm; B–C = 100 μm; D = 20 μm; G–I = 10 μm; J = 5 μm.

setae; setae short, pale brown to reddish brown, straight to flexuous, slightly tapered, blunt, 17–54 × 3–6 μm. Ascomatal wall 19–36 μm thick, of 3–6 layers of cells that are dark brown and thick-walled toward outside, becoming thin-

walled, hyaline toward centrum, wall slightly thicker around non-papillate ostiolum, of 5–9 layers of cells.

Asci bitunicate, 53–77 × 10–18 µm, cylindrical to broadly cylindrical with rounded apex, with short stalk, attached at base of ascomata, eight-spored. Interthecial elements arising from basal portion of ascomatal centrum, multicellular, 2.5–4 µm wide, septate, hyaline, anastomosing above asci.

Ascospores broadly ellipsoidal, 16–20.8 × 5.9–8.4 µm (ave. 19.7 × 7.4 µm, n = 63), pale to medium brown, three-septate, slightly constricted at median septum, first and third secondary septa, cells about equal in length, widest at penultimate cell, apex broadly rounded, base rounded, surface smooth, gelatinous sheath not observed.

Specimens examined: **CHINA. Gansu Province**, Kangle County, Fucheng, Zhangjian Village, on living twigs of *Picea crassifolia*, 17 April 2013, Y.M. Liang (BJFC-S462); Huguan, Ershilipu Village, on living twigs of *Picea crassifolia*, 17 April 2013, Y.M. Liang (BJFC-S463); Minglu, Mingguan Village, on dying twigs of *Picea crassifolia* 17 April 2013, Y.M. Liang (BJFC-S464, BJFC-S465); Xiajiazhai Nursery, on dying twigs of *Picea crassifolia*, 31 July 2013, Y.M. Liang (BJFC-S466; culture BJFC-LSH01; GenBank KF668244, KF668245); Suji Town, on living twigs of *Picea crassifolia*, 31 July 2013, Y.M. Liang (BJFC-S467; culture BJFC-LSH02; GenBank KF668246); Sanchaping Forestry Farm, on dying twigs of Picea crassifolia, 1 August 2013, Y.M. Liang (BJFC-S468; culture BJFC-LSH03; GenBank KF668247).

Phylogeny

The SSU nrRNA region dataset included sequences from 44 fungal specimens representing 40 taxa. The dataset had an aligned length of 1,056 characters of which 836 characters are constant, 86 are variable and parsimony-uninformative, and 134 are parsimony-informative. Maximum parsimony analysis yielded 52 equally parsimonious trees (TL = 391, CI = 0.659, RI = 0.833, RC = 0.549). Best model for SSU nrRNA region was estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The Bayesian analysis resulted in the same topology with an average standard deviation of split frequencies = 0.008946.

The fungus was readily recognized as a member of *Phaeosphaeriaceae* (*Pleosporales*) based on a primary BLAST search of GenBank using the rRNA ITS1-5.8S-ITS2 region. The phylogeny inferred from SSU nrDNA region sequences resulted in seven major clades for the 44 species (Fig. 2). Three isolates of *Setomelanomma holmii* formed a strongly supported clade (MP = 98%; 1.00 BBP = 1.00) with the AF525675 sequence from the *S. holmii* holotype strain PC99.4334.

Discussion

Our collections are identified as *Setomelanomma holmii* based on morphological observations and molecular phylogenetic analyses.

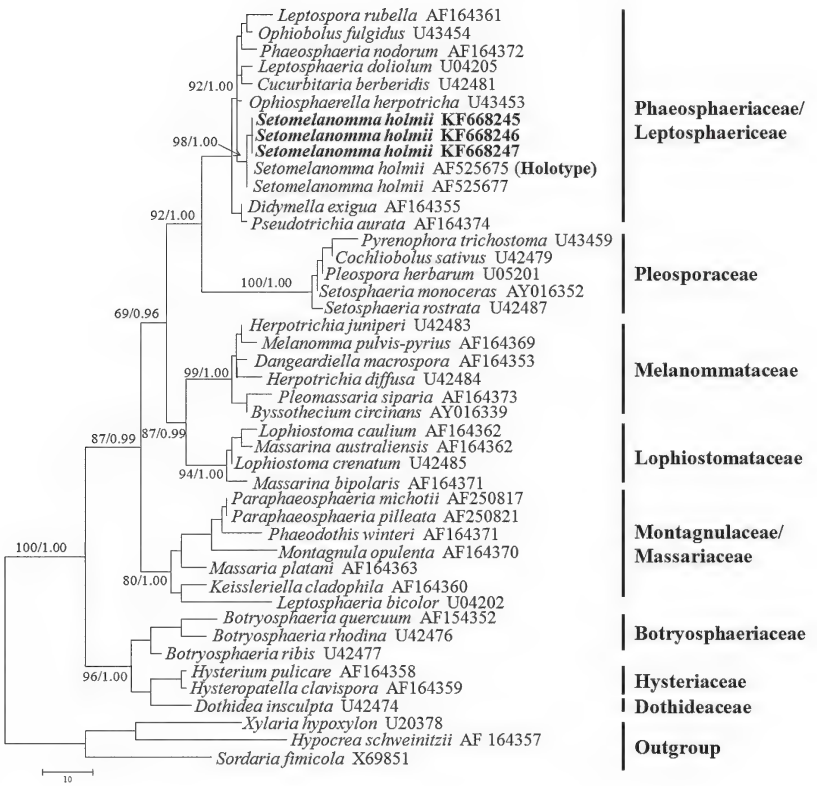

FIGURE 2. One of the 52 parsimonious trees illustrating the phylogeny of *Setomelanomma holmii* and related species based on nSSU sequences. Parsimony bootstrap proportions greater than 50% (before the slash marks) and Bayesian posterior probabilities greater than 0.95 (after the slash marks) are indicated along branches.

Morphologically, they are characterized by setaceous ascomata, bitunicate asci, and broadly ellipsoidal 3-septate ascospores measured 16–20.8 × 5.9–8.4 μm.

Setomelanomma was established as a monotypic genus based on *S. holmii* (Morelet 1980) and belongs to the *Phaeosphaeriaceae*. It can be distinguished from other genera of *Phaeosphaeriaceae* by the brown, three-septate ascospores, setose ascomata, and occurrence on conifers (Barr 1992). The genus is morphologically similar to *Kalmusia* Niessl and *Phaeosphaeria* I. Miyake to which it appears related (Hedjaroude 1968, Rossman et al. 2002, Zhang et al. 2012). *Kalmusia* differs in having immersed ascomata at maturity and well-developed apical papillae (Rossman et al. 2002). *Phaeosphaeria* is distinguished from *Setomelanomma* by absence of setae, causing diseases of angiosperm

hosts, and presence of coelomycetous anamorphs (Holm 1957, Hedjaroude 1968, Leuchtmann 1984).

In the phylogenetic tree (Fig. 2), the three isolates of *S. holmii* from China nested within the *Phaeosphaeriaceae*, and grouped closely with the type strain (PC99.4344) of *S. holmii*, forming a well supported lineage distinct from other species with a 92% bootstrap value and 1.00 Bayesian posterior probability.

Setomelanomma holmii causes a disease called spruce needle drop (SNEED). It has been reported in association with *Picea pungens* Engelm. and *P. glauca* (Moench) Voss (Morelet 1980; Rossman et al. 2002) in France and North America. This is the first report of this species in China, and the first report on the new host *P. crassifolia*. Although Kim et al. (2011) reported *S. holmii* from soybean paste in Korea, this report was based solely on a sequence similarity.

Acknowledgments

We express our gratitude to Dr. Amy Rossman (Systematic Mycology and Microbiology Laboratory, USDA-ARS, MD, USA) and Dr. Yi-Jian Yao (Institute of Microbiology of the Chinese Academy of Sciences, Beijing, China) who reviewed the manuscript. The research was financed by the National Natural Science Foundation of China (No.31170603).

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Thalloloma ochroleucum (*Graphidaceae*), a new species from Guizhou, China

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Abstract — *Thalloloma ochroleucum* is described as a new species from Guizhou province in southern China. It is characterized by subimmersed lirellae, open discs with pale orange pruina, transversely septate hyaline ascospores, and the presence of norstictic acid.

Key words — taxonomy, lichenized fungi, *Ostropales*, *Ostropomycetidae*

Introduction

In recent years, many systematic and taxonomic changes have occurred in the *Graphidaceae* (*Ostropales*, *Ostropomycetidae*). Staiger (2002) revised the spore-based generic system established by Müller Argoviensis (1880, 1882) in the family and reintroduced several genera with revised concepts, including *Thalloloma* Trevis. *Thalloloma* is characterized by a corticolous thallus, lirelliform apothecia, uncarbonised proper exciples, and hyaline ascospores (septate with lenticular locules or muriform), reacting I+ blue. *Thalloloma ochroleucum* is described here as new to science.

Materials & methods

The specimens are deposited in the Herbarium Mycologicum Academiae Sinicae – Lichenes (HMAS-L). A dissecting microscope (Tech XTS-30D with Canon 600D camera) and a light microscope (Olympus SZ-51) were used for the morphological and anatomical studies. Measurements and illustrations were taken from manual cross-sections of fruit bodies in tap water. Amyloidity of the ascospores was tested using Lugol's solution. Spot tests with KOH (20%) were performed on the thallus surface and on thin thallus sections. The lichen substances were detected and identified by thin-layer chromatography (TLC) (Culberson & Kristensson 1970, Culberson 1972, Orange et al. 2001).

Taxonomy

Thalloloma ochroleucum Z.F. Jia & Kalb, **sp. nov.** Pl. 1

Fungal Name FN570059

Differs from *Thalloloma deplanatum* by its production of norstictic acid and its longer ascospores.

Type: China, Guizhou province, Tongren City, Mt. Fanjing, Daling, 27°55′N 108°41′E, alt. 1800 m, on bark of *Rhododendron rufum* Batalin (*Ericaceae*), 22.VIII. 1963. coll. J.C. Wei 0474 (**Holotype**, HMAS-L 047744).

Etymology: Latin *ochroleucus*, a reference to the ochre-colored disc.

Thallus corticolous, crustose, pale white to yellowish, thin, smooth, tightly attached to the substratum, lacking isidia and soredia. Apothecia lirelliform, numerous, short to elongate, open, simple, subimmersed, 0.8–2.5 mm long, 0.3–0.6 mm wide, with thick thalline margin, not striate, scattered over the thallus, disc opened with orange pruina. Proper exciple inconspicuous, uncarbonised. Epithecium 8–10 μm thick, brown. Hymenium colorless, not inspersed, 150–180 μm high, I–. Hypothecium brownish, 5–15 μm high. Paraphyses simple, 1–2 μm wide, apices becoming broad, brownish. Asci

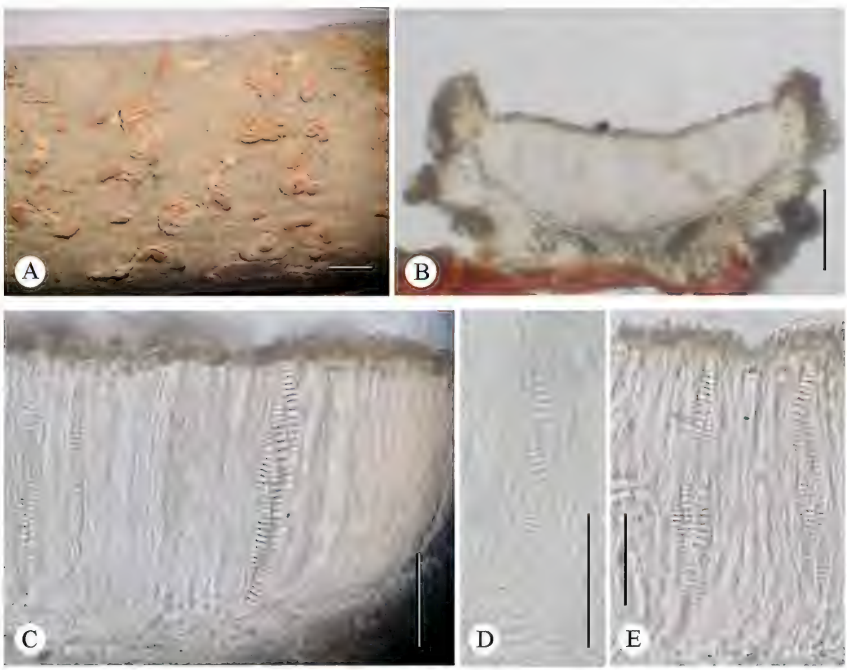

Plate 1. *Thalloloma ochroleucum* (holotype). A. Thallus on bark. B. Cross section of an apothecium. C. Asci containing ascospores. D. Immature ascospores in an ascus. E. Mature ascospores in asci. Scales: A = 1 mm; B = 100 μm; C, D = 50 μm.

cylindrical to clavate, 95–110 × 15–20 µm, 8-spored. Ascospores hyaline, narrowly ellipsoidal, transversely septate, 15–18-locular, 40–80 × 7.5–12 µm, I+ bluish.

Chemistry: C–, K+ yellow to brown, P+ yellow; norstictic acid detected by TLC.

Distribution & ecology: *Thalloloma ochroleucum* is present in Mt. Fanjing (Guizhou province) within a subtropical rainforest in southern China. The species was found on bark of *Rhododendron rufum* accompanied by *Graphis* spp. such as *G. hossei* Vain., *G. vittata* Müll. Arg., and *G. librata* C. Knight.

Additional specimen examined: **China**, **Guizhou province**, Tongren City, Mt. Fanjing, Zhangjia Dam, alt. 1000 m, on bark, 16.VIII. 1963. coll. J.C. Wei 0204 (HMAS-L 047741).

Comments: *Thalloloma ochroleucum* resembles *T. deplanatum* (Nyl.) Staiger, which also has transversely septate ascospores and open discs, but differs by its shorter thick-walled ascospores, connective paraphyses, and absence of norstictic acid (Staiger 2002). The new species is readily distinguished from *T. microsporum* Z.F. Jia & J.C. Wei described from China, which has red apothecia and smaller ascospores (15.5–20 × 5.5–8.0 µm; Jia & Wei 2009).

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A new species of *Marasmius* sect. *Sicci* from India

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Abstract — The marasmioid fungus *Marasmius midnapurensis* (*Marasmiaceae*, *Basidiomycota*) is described as a new species from India. Analysis of the internal transcribed spacer-1 region (ITS1) of the nuclear ribosomal RNA gene suggests that *M. midnapurensis* is phylogenetically distinct from closely related species and confirms its position within *Marasmius* sect. *Sicci*. Data on macro- and microscopic characters, habitat and comparisons with morphologically similar species are provided.

Key words — *Agaricomycetes*, biodiversity, phylogeny, taxonomy

Introduction

Marasmius Fr. (*Marasmiaceae*, *Agaricomycetes*, *Basidiomycota*), as traditionally accepted by Singer (1986), is polyphyletic (Wilson & Desjardin 2005). Based on the nLSU rDNA sequences, Wilson & Desjardin (2005) restricted the genus to a monophyletic lineage containing only sections *Marasmius, Sicci, Hygrometrici, Globulares, Neosessiles, Scotophysini,* and *Leveilleani* where taxa in *Sicci* and *Globulares* form a large joint clade (Wannathes et al. 2009). *Marasmius* sect. *Sicci* includes species characterized by a hymeniform pileipellis of broom cells of the *Siccus*-type and dextrinoid hyphae (Singer 1958, 1986).

Sixty-eight species and one variety have been reported thus far in India (Manjula 1983, Manimohan & Leelavathy 1989, Bilgrami et al. 1991). In the state of West Bengal only nine species of the genus have been reported: *Marasmius consocius* Berk., *M. erythropus* (Pers.) Fr., and *M. burkillii* (Massee) Manjula from the Darjeeling area (Berkeley 1851, Massee 1910); *M. pangerangensis* Henn., *M. campanella* Holterm., and *M. haematocephalus* (Mont.) Fr. from

Calcutta (Bose 1949, Bose & Chatterjee 1950, Roy 1953); *M. umbrinus* Pegler from Bankura district (Ray & Samajpati 1979); and *M. androsaceus* (L.) Fr. and *M. siccus* (Schwein.) Fr. from the lateritic region of West Bengal (Pradhan et al. 2011). All of these records should be revised in the light of modern taxonomic concepts and the many new species that have been described, especially from tropical regions.

The present paper describes *Marasmius midnapurensis*, a new fungal species from West Bengal.

Materials & methods

Basidiomata sampling and morphological studies

Basidiocarps of *Marasmius midnapurensis* were collected in 2011 during field trips to the state of West Bengal, India. Their morphology and ecology were noted and colour photographs were taken in the field. Microscopic features were obtained from dried material by mounting free-hand sections in 5% potassium hydroxide (KOH), Melzer's reagent, Congo red, or lactophenol-cotton blue and examination using a Carl Zeiss AX10 Imager A1 phase contrast microscope. Colour terms follow the British Fungus Flora Colour Chart (Anonymous 1969).The terms used to describe lamellae spacing are L for number of lamellae and l for number of lamellulae between two lamellae. Spore statistics include: X_m, the arithmetic mean of the spore length by spore width (± standard deviation) for n spores measured in a single specimen; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, total number of spores measured; s, the number of specimens. The holotype collection has been deposited in the Calcutta University Herbarium (CUH).

DNA extraction, Polymerase Chain Reaction, and sequencing

Genomic DNA was extracted from dried (50°C) herbarium tissue (10–50 mg) using the 'Fungal gDNA Mini Kit' (Xcelris Genomics, Ahmedabad, India). The internal transcribed spacer-1 region (ITS1) was amplified using ITS1-F (Gardes & Bruns 1993) and ITS2 (White et al. 1990) primer pair. A hot start of 2 min at 94°C was followed by 30 cycles consisting of 30 s at 94°C, 1 min at 56°C, 1 min at 72°C, and a final elongation step of 5 min at 72°C. PCR products were checked on 2% agarose gel stained with ethidium bromide. PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and sequencing was done using Sanger methods. The obtained sequence generated was submitted to GenBank.

Phylogenetic analysis

Our new ITS1 sequence was submitted to GenBank (www.ncbi.nlm.nih.gov), and related *Marasmius* sequences were identified by a BLASTn search (http://blast.ncbi.nlm.nih.gov/). Sequences used in the phylogenetic analysis are indicated in Fig. 3, with *Mycena pura* (Pers.) P. Kumm. and *Marasmius rotula* (Scop.) Fr. as outgroup.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein

1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor 1969) and represent the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 142 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Taxonomy

Marasmius midnapurensis A.K. Dutta, P. Pradhan & K. Acharya, **sp. nov.** Figs 1, 2

MycoBank MB809410

Differs from *Marasmius jasminodorus* by its lighter colored and striate pileus, creamy lamellae forked towards the margin, non-strigose stipe base with whitish mycelium, slightly larger basidiospores, and the absence of a sweetish odor.

Type: India, West Bengal, Midnapur District, Ramnagar, Kasaphaltala, 21°43′29.9″N 87°31′36.6″E, 10 m asl., on dried *Acacia* leaves and on wood, 11 Aug. 2011, A.K. Dutta, P. Pradhan & K. Acharya (**Holotype,** CUH AMT002; GenBank, KF682470).

Etymology: specific epithet refers to the type locality.

Pileus 22–27 mm diam., broadly convex, sometimes umbonate, smooth, viscid when moist, light brown to light greyish brown with irregular light yellowish brown patches in the center, hygrophanous, smooth, striate. Context ≤0.8 mm thick, creamy, not changing colour when exposed. Lamellae L = 13–14, l = 3–4, adnexed, subdistant, forked towards the margin, creamy, regular, ≤4 mm broad, spacing ≤5 mm, margin even, concolorous. Collarium absent. Stipe 53–65 mm long, 2 mm broad overall, central, cylindrical, dark brick brown lower, creamy in upper part, equal, hollow, cartilaginous, strict to curved at lower portion, dry, smooth, flesh concolorous with the pileus, non-insititious, base covered with whitish mycelium. Odor and taste mild.

Basidiospores (10.7–)11.08–12.2(–15) × (3.5–)3.9–4.3(–4.7) µm [X_m = 11.57 ± 1.04 × 3.9 ± 0.39, Q = 2.5–3.5, Q_m = 3 ± 0.24, n = 150 spores (30 spores each among the collected 5 basidiocarps), s = 1 specimen], ellipsoid, slightly curved in profile, smooth, hyaline, inamyloid, thin-walled. Basidia (17.9–)23.2–23.6 (–24.4) × 4.7–6.8(–7.9) µm, clavate, hyaline, 4-spored, sterigmata 3.2–3.6 µm long. Basidioles 17.9–21.5 × 6.8–7.2 µm, fusoid to clavate, hyaline. Cheilocystidia common, of *Siccus*-type broom cells, main body 13.6–17.9 × 7.2–10 µm, cylindrical to clavate, hyaline, inamyloid, thin- to thick-walled, apical setulae 3.9–6.8(–10) × 1.4–1.8 µm, irregular in outline, obtuse to subacute, yellow to brownish yellow, thick-walled. Pleurocystidia absent. Pileipellis hymeniform, mottled, composed of *Siccus*-type broom cells, main

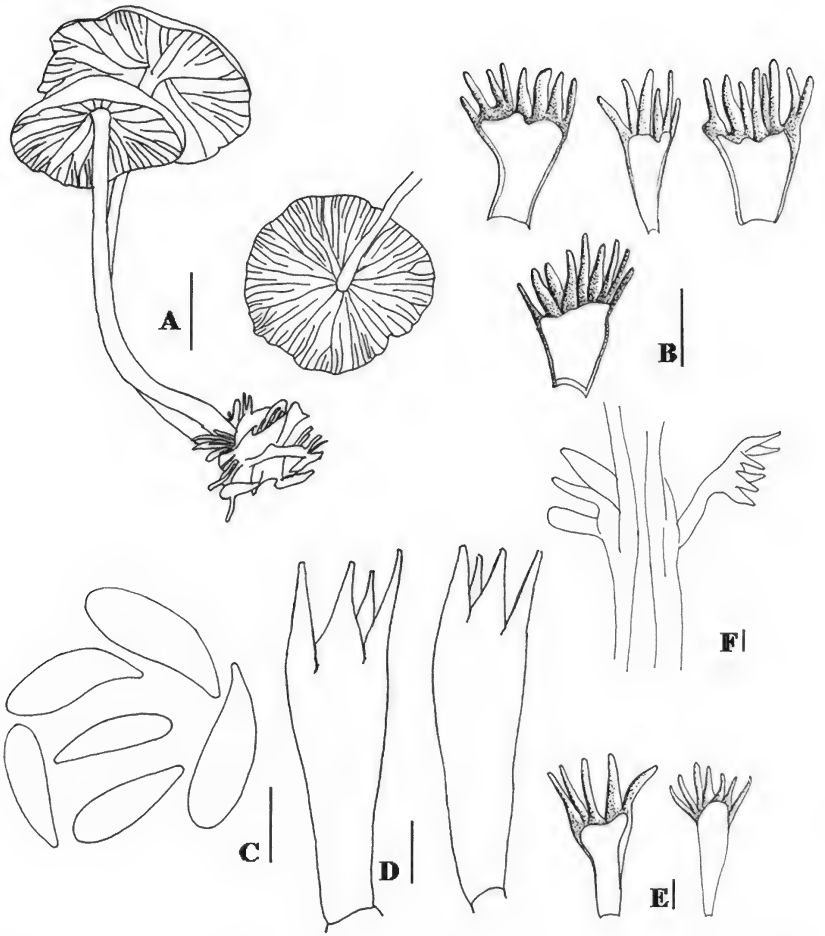

Figure 1. *Marasmius midnapurensis* (holotype): A. Basidiomes. B. *Siccus*-type cells of pileipellis. C. Basidiospores. D. Basidium. E. Cheilocystidia. F. Caulocystidia. Scale bars: A = 10 mm; B, F = 10 µm; C-E = 5 µm.

body 13.6–14.3 × (6.8–)7.1–10.3(–10.7) µm, clavate to broadly clavate, often branched, hyaline, inamyloid, thin- to thick-walled, apical setulae 3.9–6.8(10) × 1.4–1.8 µm, crowded, cylindrical to irregular in outline, obtuse to subacute, yellowish brown to brown, thick-walled, setae absent. Pileus trama interwoven, strongly dextrinoid. Lamellar trama hyphae interwoven, cylindrical to inflated, smooth, hyaline, dextrinoid, thin-walled, non-gelatinous. Stipitipellis hyphae 6–7 µm broad, parallel, yellowish brown to brown, smooth, dextrinoid, thick-walled, wall ≤0.7 µm thick, non-gelatinous.

FIGURE 2. *Marasmius midnapurensis* (CUH AMT002). Basidiomata. Scale bar = 10 mm.

STIPE TRAMA hyphae parallel, hyaline, smooth, dextrinoid, thin-walled, non-gelatinous. OLEIFEROUS HYPHAE present, ≤6.1 µm broad. CAULOCYSTIDIA composed of two types of cells: a) *Siccus*-type broom cells with main body 28–29.4 × 3.5–4.3 µm, scattered, uncommon, irregular in outline, hyaline, apical setulae 6–6.4 × 1.8–2.1 µm, conical to wavy, pale yellow, thin- to thick-walled, b) non-setulose cells (11–)28.6–42.9(–50.1) × (3.5–)3.9–4.7(–6.3) µm, abundant, cylindrical or irregular in outline, seldom branched, obtuse to subacute, hyaline, inamyloid, thin- to thick-walled. CLAMP CONNECTIONS present in all tissues.

Molecular & phylogenetic analysis

The amplified fragment of *M. midnapurensis* with the combination of primer set ITS1-F (forward) and ITS2 (reverse) produced 259 bp long stretches including the 226 bp ITS1 region. BLAST analyses with our *M. midnapurensis* sequences recovered *Marasmius* sequences, with the highest similarity shown by *M. jasminodorus* Wannathes et al. (92%), *M. araucariae* var. *siccipes* Desjardin et al. (89%), *M. aurantioferrugineus* Hongo (87%), *M.* cf. *cladophyllus* Berk. (87%), and *M. purpureostriatus* Hongo (84%).

Phylogenetic analysis of the ITS1 region inferred by the neighbor-joining method strongly supports *M. midnapurensis* as a distinct species within *Marasmius* and clusters *M. midnapurensis* and the seven other in-group

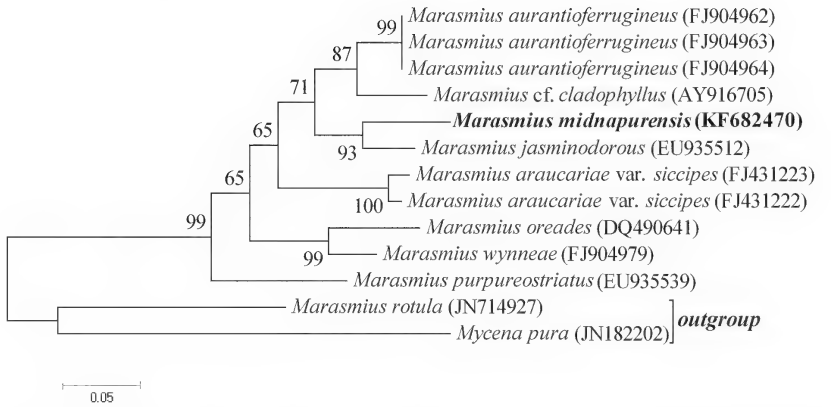

FIGURE 3. Phylogram showing relationship of *Marasmius midnapurensis* to closely related species of *Marasmius* based on internal transcribed spacer-1 (ITS1) sequences of rDNA. *Marasmius midnapurensis* is placed in bold font to highlight its phylogenetic position in the tree.

Marasmius spp. in a clade with 99% bootstrap support. Although *M. midnapurensis* forms a distinct branch sister to *M. jasminodorus* with 93% bootstrap support in the present study, another closely related species (*M. araucariae* var. *siccipes*, also in sect. *Sicci* ser. *Atrorubentes*) forms a separate clade with 65 % bootstrap support (FIG. 3).

Discussion

The absence of collarium and the presence of *Siccus*-type broom cells in the pileipellis, a well developed long central stipe, and adnexed lamellae suggest that *M. midnapurensis* belongs to *Marasmius* sect. *Sicci* Singer, while the presence of *Siccus*-type of broom cells combined with non-setulose cells on the stipe surface place it in the series *Atrorubentes* (Wannathes et al. 2009), where it appears to be closely related to *M. araucariae* var. *siccipes* described from Java, which differs in a more brownish orange pileus and darker lamellae (brownish orange to greyish brown), and slightly smaller basidiospores (8–12 × 3.5–4 µm; Desjardin et al. 2000).

Basidiospore shape and size, cheilocystidial and caulocystidial characters, and the absence of pleurocystidia are characters shared with *M. jasminodorus* from northern Thailand [ellipsoid basidiospores 9–14 × 3–4.5 µm with Q_m of 2.6–3.4, *Siccus*-type cheilocystidia (9–26 × 6–10 µm), caulocystidia of *Siccus*-type broom cells and cylindrical non-setulose cells, absence of pleurocystidia]. *Marasmius jasminodorus* differs macroscopically in its light brown to brownish pileus, pale yellowish white lamellae with pale brownish orange edges, a fragrant jasmine tea-like odor, and stipe base with strigose, brownish orange mycelium (Wannathes et al. 2009). Another similar species, *M. koreanus* Antonín et al.

Table 1. Comparison of *Marasmius midnapurensis* with similar *Marasmius* species.

Taxa	Pileus colour	Lamellae colour	Stipe base	Basidiospores (µm)	Cheilocystidia	Caulocystidia	Odor
M. midnapurensis	Light brown to light greyish brown; disc with irregular light yellowish brown patches	Creamy	Covered with whitish mycelium	10.7–15 × 3.5–4.7	*Siccus*-type	*Siccus*-type + non-setulose cells	Not distinctive
M. jasminodorus	Disc dark reddish brown; margin brown to brownish orange	Pale yellowish white	Strigose, mycelium brownish orange	9–14 × 3–4.5	*Siccus*-type	*Siccus*-type + non-setulose cells	Sweet, like jasmine tea
M. araucariae var. *siccipes*	Disc dark brown; margin brown to brownish orange	Brownish orange to greyish brown	Non-insititious	8–12 × 3.5–4	*Siccus*-type	*Siccus*-type + non-setulose cells	Not distinctive
M. purpureostriatus	Disc dark violet; sulcae greyish violet; elsewhere greyish yellow	Pale yellow	Non-insititious	19–30 × 4–7	Cylindrical to broadly clavate or pyriform	Absent	Not distinctive
M. aurantio-ferrugineus	Orange-ferrugineous	Pale yellowish white	Broadened	11.5–15 × 4–4.5(6)	Clavate, fusoid, subcylindrical, (sub) vesiculose	Cylindrical, (narrowly) clavate, (sub)fusoid	Not distinctive

from Republic of Korea, differs by brownish orange to reddish orange pileus with a distinctly brownish orange, rugulose centre and absence of caulocystidia (Antonín et al. 2012). All other species of *Marasmius* sect. *Sicci* described by Deng et al. (2012) from China differ from *M. midnapurensis* by the presence of setae on the pileus and only a single type of caulocystidia. In addition to the morphological differences (Table 1), the ITS sequence analysis clearly separates *M. midnapurensis* from *M. jasminodorus*, *M. aurantioferrugineus*, *M.* cf. *cladophyllus*, and *M. purpureostriatus*. Our findings support Tan et al. (2009) and Wannathes et al. (2009), who found that molecular data do not support morphologically delimited concepts within *Marasmius* groups. The objective of our study was to confirm the taxonomical position of *M. midnapurensis* (sect. *Sicci* ser. *Atrorubentes*) using ITS rDNA sequence data.

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The authors are grateful to Armin Mešić (Ruđer Bošković Institute, Zagreb, Croatia), T.K. Arun Kumar (Zamorin's Guruvayurapan College, Kerala, India), and Alfredo Justo (Clark University, Worcester, United States) for their critical reviews of the manuscript. Thanks are also owed to Vladimír Antonín (Brno, Czech Republic) for his valuable suggestions on the allied species of the newly described taxa and Patinjareveettil Manimohan (University of Calicut, Malappuram, India) for his guidance during manuscript preparation. This paper appears through financial support by the Department of Environment, Government of West Bengal, India.

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A new species of *Arachnophora* from submerged wood in the Amazon rainforest, Brazil

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Abstract — *Arachnophora combuensis* sp. nov. is described and illustrated from specimens collected on submerged wood from Combu Island (Belém municipality, Pará, Brazil) in the Amazon rainforest. The fungus is characterized by simple pale brown conidiophores, monoblastic percurrently extending conidiogenous cells, and staurosporous conidia with a pale brown basal cell and 2–3 dark brown central cells that bear 1–3 conical pale brown arms. The new species is compared with morphologically similar taxa.

Key words — taxonomy, freshwater fungi, tropics

Introduction

During a mycological survey of conidial fungi occurring on submerged decaying plant materials from the Amazon rainforest in Brazil, an interesting fungus was collected on submerged wood. Its conidiogenesis and conidial features clearly suggest placement within the genus *Arachnophora* Hennebert (Hennebert 1963). However, the fungus shows remarkable differences from all previously described *Arachnophora* species and therefore is described as new to science.

Material & methods

Samples of submerged litter were placed in paper and plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements were taken at ×1000.

Micrographs were obtained with an Olympus microscope (model BX51) equipped with bright field and Nomarski interference optics. The type specimens are deposited in the Herbarium of Universidade Estadual de Feira de Santana (HUEFS).

Taxonomy

Arachnophora combuensis J.S. Monteiro, R.F. Castañeda & Gusmão, **sp. nov.** Fig. 1

MycoBank MB 807635

Differs from *Arachnophora crassa* by its wider conidial central body and fewer, larger conidial arms and from *A. pulneyensis* by its wider conidial central body separated from the conidiogenous cell by a basal cell.

Type: Brazil, Pará State, Belém, Área de Proteção Ambiental Ilha do Combu, 1°25'S 48°27′ W, on submerged wood, 10 Jan. 2013, coll. J.S. Monteiro (**Holotype**: HUEFS 196432).

Etymology: Latin *combuensis* refers to the island where the type specimen was collected.

Colonies on the natural substrate effuse, hairy, dark brown. Mycelium superficial and immersed. Hyphae branched, septate, smooth, pale brown, 2–4 µm wide. Conidiophores macronematous, mononematous, simple or with one branch, erect, straight or slightly flexuous, cylindrical, 3–4-septate, smooth, pale brown, 87.5–225 × 5–7.5 µm; branches cylindrical, 1–2-septate, smooth, pale brown, 27.5–60 × 6–7.5 µm. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, with enteroblastic percurrent extensions, smooth, pale brown. Conidial secession rhexolytic. Conidia acrogenous, solitary, staurosporous, 42–50(–60) × 13–16 µm, composed of a basal cell that is conical, smooth, pale brown, 7–14 × 5–11 µm, with a tapering base bearing remains of the conidiogenous cells, 2–3 µm wide; 1–2(–3) central cells that are dark brown, smooth, 7–21 × 9–16 µm; 1–3(–4) arms that are conical, 1-septate, 12–30 × 5–11 µm, with a pale brown basal cell, 6–15 × 5–11 µm and a pale brown distal cell, 7–19 × 3–6 µm. Synanamorph *Selenosporella*-like, present at the tip of conidial arms, producing conidia sympodially, aseptate, fusiform, straight, hyaline, 3–5 × 0.5–1 µm.

Additional specimen examined: **BRAZIL, Pará**, Área de Proteção Ambiental Ilha do Combu, 1°25'S 48°27′W, on submerged wood, 10 Jan. 2013, coll. J.S. Monteiro (HUEFS 196433).

Note: *Arachnophora* is typified by *A. fagicola* Hennebert, which was collected from decaying cupules of *Fagus sylvatica* in Belgium (Hennebert 1963). The genus comprises nine species characterized by monoblastic integrated percurrently extending conidiogenous cells that produce staurosporous conidia with dark central cells and paler septate incurved arms (Révay & Gönczöl 1989, Castañeda-Ruiz et al. 1996, 1997, Castañeda-Ruiz & Guarro 1998, Pratibha et al. 2011, Leão-Ferreira et al. 2013, Ma et al. 2013). Among the described

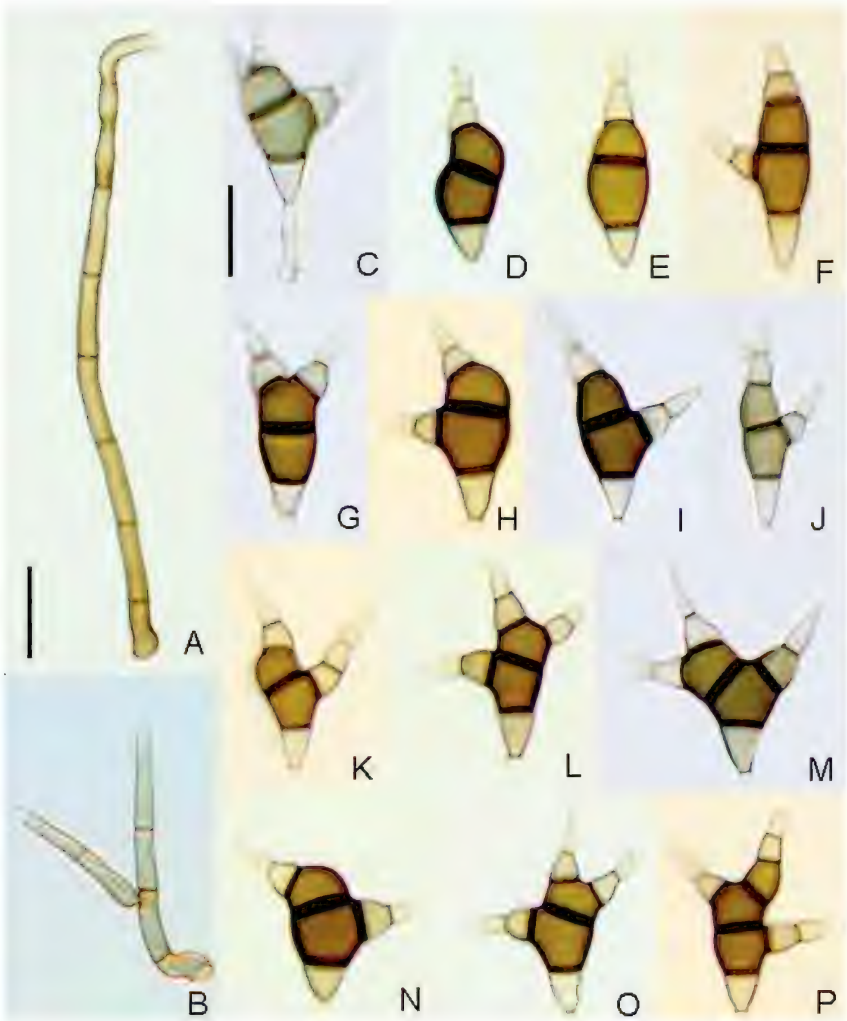

FIG. 1. *Arachnophora combuensis* (holotype, HUEFS 196432). A–B. Conidiophores. C. Conidiogenous cell attached to a conidium. D–P. Conidia. Scale bars: A–B = 30 µm; C–P = 20 µm.

species only *Arachnophora crassa* Révay & J. Gönczöl and *A. pulneyensis* (Subram. & Bhat) R.F. Castañeda are morphologically similar to *A. combuensis*. *Arachnophora crassa* is distinguished by conidia with 5–7 arms measuring 9–14.5 × 5–6.5 µm and a two-celled central body of 16–22.5 × 8–9.5 µm (Révay & Gönczöl 1989). *Arachnophora pulneyensis* differs from *A. combuensis* by the

sizes of the central body (16–24 × 6.5–8 µm) and conidial arms (14 × 3–5 µm) and the absence of a basal cell (Castañeda-Ruiz et al. 1996).

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Xiuguozhangia, a new genus of microfungi to accommodate five *Piricaudiopsis* species

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Abstract — *Xiuguozhangia* gen. nov. is established to accommodate five species described in *Piricaudiopsis* but characterized by holoblastic conidial ontogeny. Five new combinations are proposed: *Xiuguozhangia appendiculata*, *X. indica*, *X. punicae*, *X. rhaphidophorae*, and *X. rosae*. Notes and illustrations on the type species of *Piricaudiopsis*, *P. elegans*, are also provided.

Key words — asexual fungi, systematics, leaf litter

Piricaudiopsis J. Mena & Mercado, typified by *P. elegans* J. Mena & Mercado (Mena Portales & Mercado Sierra 1987), is characterized by conidiophores that are macronematous, mononematous, unbranched, erect, cylindrical, and dark brown and conidiogenous cells that are monotretic, integrated, terminal and discrete, intercalary, doliiform, and subspherical to rather lageniform (Fig. 1). Conidial ontogeny is enteroblastic; the conidia are dictyoseptate, with several compact rows of cells, somewhat complanate, brown, verrucose towards the base, verruculose or smooth towards the distal cells; 4–7 of the apical cells produce a divergent, straight to slightly curved arm.

Five additional species have been described in *Piricaudiopsis*: *P. appendiculata* (Bhat & Kendrick 1993); *P. indica* (Sureshkumar et al. 2005); and *P. punicae*, *P. rhaphidophorae*, and *P. rosae* (Zhang et al. 2009). All five of these species have holoblastic conidial ontogeny (in contrast to the enteroblastic conidial

ontogeny of *P. elegans*). We therefore propose a new genus, to which we transfer the five holoblastic species.

Taxonomy

Fig 1. ***Piricaudiopsis elegans*** (INIFAT C06/70). A. Conidia. B. Conidiogenous cells. Fig 2. ***Xiuguozhangia rosae*** (HSAUPVII$_{0\text{-}KAI}$1092). Conidiogenous cells and conidia.

Xiuguozhangia K. Zhang, R.F. Castañeda, Jian Ma & L.G. Ma, **gen. nov.**

MycoBank MB 807641

Differs from *Piricaudiopsis* by its holoblastic conidial ontogeny.

Type species: *Xiuguozhangia rosae* (K. Zhang & X.G. Zhang) K. Zhang & R. F. Castañeda

Etymology: Latin *Xiuguozhangia*, dedicated to Prof. Xiu Guo Zhang (Shandong Agricultural University, China) for his contribution to microfungal taxonomy.

Colonies on the natural substrate effuse, hairy, velutinous, brown to dark brown. Mycelium mostly superficial immersed. Conidiophores distinct, single, cylindrical, erect, straight or flexuous, unbranched or moderately branched, multiseptate, brown to dark brown below, mid to pale brown toward

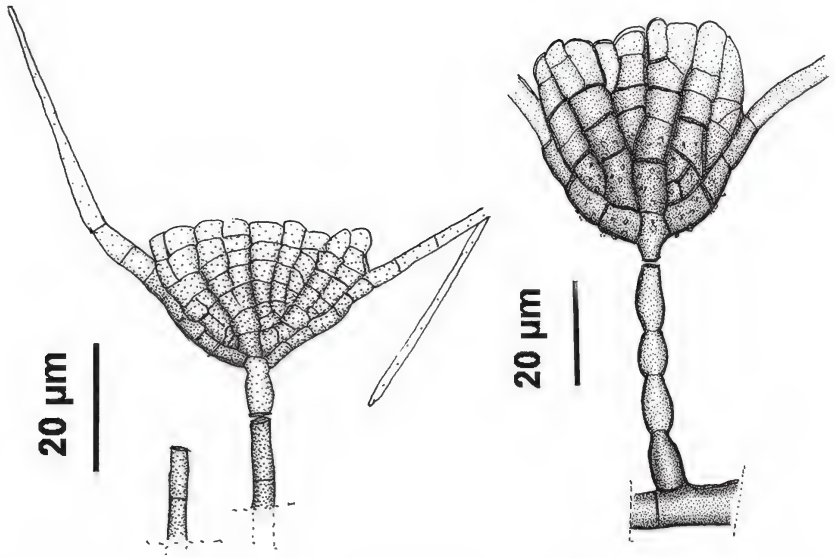

Fig 3. ***Xiuguozhangia appendiculata*** (DAOM 214610). Conidiogenous cell and conidium. Fig 4. ***Xiuguozhangia indica*** (ex HCIO-45498). Conidiogenous cell and conidium. Redrawn from original publication.

the apex, smooth or verruculose. Conidiogenous cells monoblastic, mostly discrete, few, terminal, integrated, lageniform, determinate, or indeterminate with several enteroblastic percurrent elongations, pale brown. Conidiogenous loci flat, truncated. Conidial ontogeny holoblastic. Conidial secession schizolytic. Conidia solitary, acropleurogenous, dictyoseptate, campanulate, cheiroid, composed of several compact rows of cells, that form a compact cluster after successive ± dichotomous branchings, brown, verrucose towards the base, verruculose or smooth towards the distal cells; 0–4 of the apical cells may produce a slender, divergent, straight to slightly curved, septate, pale brown, appendage.

Xiuguozhangia rosae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, **comb. nov.** Fig. 2

MycoBank MB 807642

≡ *Piricaudiopsis rosae* K. Zhang & X.G. Zhang, Mycologia 101(3): 419 (2009).

Xiuguozhangia appendiculata (Bhat & W.B. Kendr.) K. Zhang & R.F. Castañeda, **comb. nov.** Fig. 3

MycoBank MB 807643

≡ *Piricaudiopsis appendiculata* Bhat & W.B. Kendr., Mycotaxon 49: 62 (1993).

Fig 5. ***Xiuguozhangia punicae*** (HSAUPVII$_{0\text{-}KAI}$0432)Conidiophore, conidiogenous cells and conidia. Fig 6. ***Xiuguozhangia rhaphidophorae*** (HSAUPVII$_{0\text{-}KAI}$1417). A. Conidia. B–C. Conidiophores, conidiogenous cells and conidia.

Xiuguozhangia indica (Sharath, Sureshk., Kunwar & Manohar.) K. Zhang & R.F. Castañeda, **comb. nov.** Fig. 4

MycoBank MB 807644

≡ *Piricaudiopsis indica* Sharath, Sureshk., Kunwar & Manohar., Mycotaxon 92: 280 (2005).

Xiuguozhangia punicae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, **comb. nov.** Fig. 5

MycoBank MB 807646

≡ *Piricaudiopsis punicae* K. Zhang & X.G. Zhang, Mycologia 101(3): 417 (2009).

Xiuguozhangia rhaphidophorae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, **comb. nov.** Fig. 6

MycoBank MB 807645

≡ *Piricaudiopsis rhaphidophorae* K. Zhang & X.G. Zhang, Mycologia 101(3): 420 (2009).

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Hymenochaete in China. 8. *H. biformisetosa* sp. nov. with a key to species with denticulate setae

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Abstract — *Hymenochaete biformisetosa*, a new species from Yunnan Province in southwestern China, is described and illustrated. It is characterized by thick, resupinate basidiocarps, two types of setae (sometimes denticulate), and ellipsoid to broadly ellipsoid basidiospores. The new species is similar to *H. spinulosetosa* and *H. denticulata* but with morphological and molecular (ITS rDNA sequences) differences. An identification key to nine species of *Hymenochaete* with denticulate setae is provided.

Key words — *Hymenochaetaceae*, phylogeny, taxonomy, wood-inhabiting fungi

Introduction

The diversity of wood-inhabiting fungi in Yunnan Province, southwest China, is extremely high because of abundant vascular plants and complex landforms. Although many basidiomycete species have been described from this area in recent years (Dai et al. 2002; Yuan & Dai 2008a,b; Dai & Li 2010, Dai 2011, 2012; He & Li 2011, 2012, He & Dai 2012; Zhao et al. 2013), corticioid fungi are under-collected and studied.

In 2012, an intensive investigation of wood-inhabiting fungi was carried out in the Gaoligong Mountains in northwest Yunnan Province, which is considered an important biodiversity hotspot in China. Among the approximately 100 specimens collected in this survey, an undescribed species of *Hymenochaete* was found. We describe and illustrate the new fungus as *H. biformisetosa* in our new contribution to the knowledge of *Hymenochaete* species in China.

Materials & methods

Morphological studies: Voucher specimens were deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure followed Dai (2010).

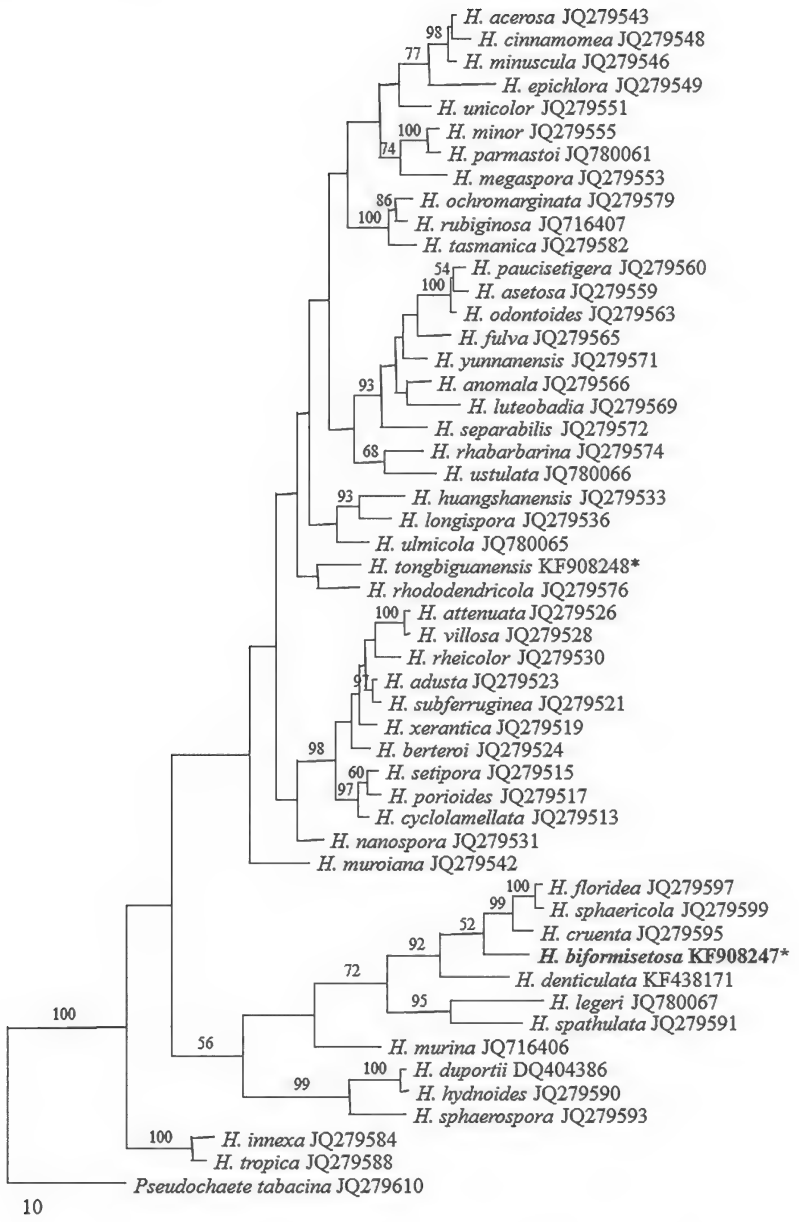


FIG. 1. Strict consensus tree obtained from Maximum Parsimony analysis of ITS sequences of *Hymenochaete* species. *Pseudochaete tabacina* was used as outgroup. Newly generated sequences are indicated by an asterisk (*). Parsimony bootstrap values >50% are shown.

In presenting the size range of spores and setae, 5% of the measurements from each end of the range are given in parentheses. Special color terms follow Petersen (1996).

DNA EXTRACTION AND SEQUENCING: A CTAB Plant genome rapid extraction kit DN14 (Aidlab Biotechnologies Co., Ltd) was used to extract DNA from dried specimens following the manufacturer's instructions. Approximately 700 base pairs of the ITS rDNA were amplified with primers ITS5 and ITS4 as follows: initial denaturation at 95 °C for 3 min, followed by 38 cycles of 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and all the newly generated sequences were deposited in GenBank.

PHYLOGENETIC ANALYSIS: The phylogeny was inferred from the ITS sequences of 51 *Hymenochaete* species. *Pseudochaete tabacina* (Sowerby) T. Wagner & M. Fisch. was selected as outgroup. The sequences were aligned using the ClustalX 1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit 7.0.5.3 (Hall 1999). Maximum parsimony analysis were performed using PAUP* 4.0b10 (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analysis were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each tree generated. Trees were figured in Treeview 1.6.6 (Page 1996).

Phylogenetic results

Two new ITS sequences were obtained in this study (GenBank accession numbers KF908247 and KF908248). The alignment of the ITS sequences of 52 taxa resulted in 764 sites with 389 parsimony informative characters. Two equally parsimonious trees were obtained from the analysis with TL = 2180, CI = 0.423, RI = 0.588, RC = 0.249 and HI = 0.577. The strict consensus tree showed that *Hymenochaete biformisetosa* formed a distinct lineage embedded in a clade with *H. denticulata* J.C. Léger & Lanq., *H. cruenta* (Pers.) Donk, *H. floridea* Berk. & Broome, and *H. sphaericola* Lloyd (FIG. 1).

Taxonomy

Hymenochaete biformisetosa Jiao Yang & S.H. He, **sp. nov.** FIGS 2–4, 8

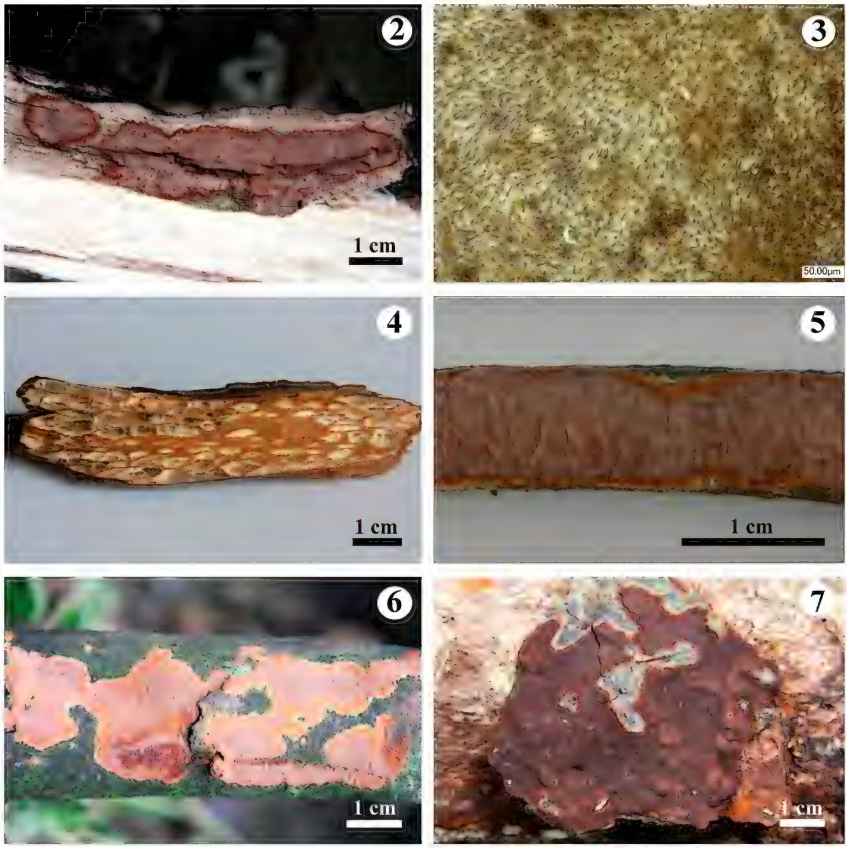
MYCOBANK MB 807093

Differs from other *Hymenochaete* species by its thick basidiocarps, two types of setae that may be denticulate, and ellipsoid to broadly ellipsoid basidiospores.

TYPE: China. Yunnan Prov., Baoshan, Gaoligongshan Nat. Res., Baihualing, on angiosperm stump, 28.X.2012, He 1445 (**holotype**, BJFC 14297; GenBank KF908247).

ETYMOLOGY: the epithet "*biformisetosa*" refers to the two types of setae.

FRUITBODY: Basidiocarps annual, effused, closely adnate, corky to woody hard, first as small round or irregular patches, later confluent up to 6 × 2 cm,



FIGS. 2–3. ***Hymenochaete biformisetosa*** (holotype): 2. Basidiocarps. 3. Close-up of hymenophore surface. 4. Wood decayed by *H. biformisetosa*. FIGS. 5–7. **Basidiocarps of other *Hymenochaete* species with denticulate setae**: 5. *H. denticulata* (He 1271). 6. *H. separabilis* (He 271). 7. *H. tongbiguanensis* (He 1552).

0.15–2.5 mm thick in section. Hymenophore smooth, grayish brown to fawn, not cracked when young, becoming pale mouse-gray to light vinaceous gray, deeply and sparsely cracked when mature; margin thinning out, reddish brown to orange-brown, distinct, up to 1 mm wide when juvenile, becoming fuscous or concolorous with hymenophore surface, narrower with age.

HYPHAL STRUCTURE: Hyphal system subdimitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in potassium hydroxide.

SUBICULUM: Tomentum and cortex absent. Hyphal layer absent or sometimes present, consist of interwoven hyphae. Generative hyphae rare,

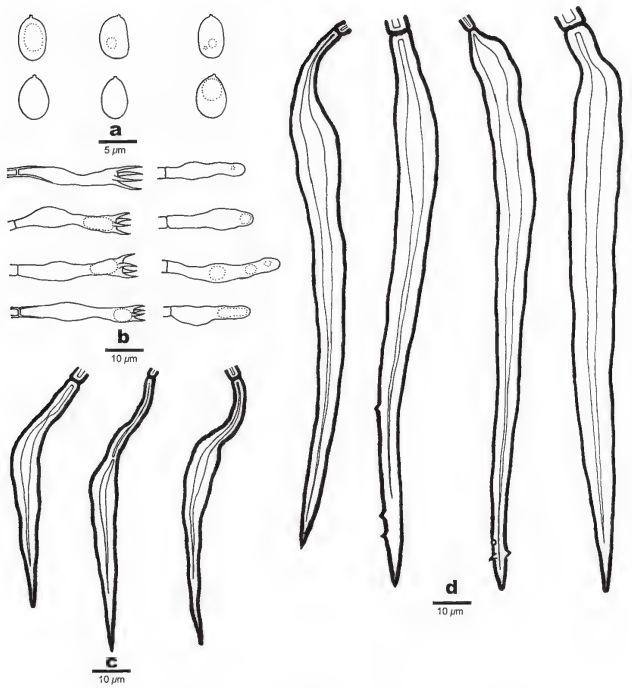


Fig. 8. *Hymenochaete biformisetosa* (holotype):
a. Basidiospores. b. Basidia and basidioles. c. Small setae. d. Large setae.

hyaline to yellowish brown, thin- to slightly thick-walled with a wide lumen, septate, moderately branched, 2–4 μm in diam. Skeletoid hyphae predominant, reddish brown, distinctly thick-walled with a narrow lumen, some parts nearly solid, usually developing into setal hyphae with acute apices, 3–4.5 μm in diam.

Stratified hymenium: Hyphae similar to those in subiculum, reddish brown, thick-walled, agglutinated, interwoven. Setal layer thick, composed of several to many rows of overlapping setae. Setae of two types (1) large, numerous, subulate, reddish brown, with acute tips, projecting up to 90 μm above the hymenium, (70–)80–130(–140) × (8–)9–14(–16) μm, often with crystals loosely attached to the projected part when mature; (2) small, numerous, subulate, reddish brown, with acute tips, some with a hyphal tail, usually not protruding, (30–)35–50(–60) × 4–8 μm. Both types of setae sometimes with 1–3 conical spines near apex; spines up to 2 μm long. Cystidia and hyphidia absent. Basidia subcylindrical to subclavate, usually restricted in central part, with four sterigmata and a simple septum at base, 20–25 × 3–5 μm; basidioles similar to basidia in shape but shorter.

Spores: Basidiospores ellipsoid to broadly ellipsoid, hyaline, thin-walled, smooth, sometimes containing one guttule, inamyloid and nondextrinoid in Melzer's reagent, acyanophilous in cotton blue, (4.1–)4.3–6(–6.5) × (2.8–)3–4.2 (–4.5) µm, mean spore length = 5.2 µm, mean spore width = 3.5 µm, length/width = 1.5 (60 spores of one specimen were measured).

Type of rot: Causes a white pocket rot of angiosperm wood.

Additional specimens examined: *Hymenochaete spinulosetosa*: **VENEZUELA. Merida Prov.**, Monte Zerpa, on dead hardwood, 29.I.2001, Ryvarden 43707 (isotype, TAAM 202926).

Hymenochaete denticulata: **CHINA. Guangxi Autonomous Region**, Longzhou County, Nonggang Nat. Res., on fallen angiosperm twig, 21.VII.2012, He 1271 (BJFC 14122; Fig. 5).

Hymenochaete separabilis: **CHINA. Anhui Prov.**, Huangshan County, Huangshan Nat. Res., on fallen angiosperm trunk, 21.X.2010, He 460 (BJFC 9938; Fig. 6).

Hymenochaete tongbiguanensis: **CHINA. Yunnan Prov.**, Ruili, on fallen angiosperm branch (paratype, HMAS 84867); Longchuan County, Tongbiguan Nat. Res., on angiosperm stump, 31.X.2012, He 1497 (BJFC 14352). **Hainan Prov.**, Lingshui County, Diaoluoshan Nat. Res., on angiosperm stump, 10.XI.2012, He 1552 (BJFC 14407; GenBank KF908248; Fig. 7).

Discussion

Hymenochaete biformisetosa can be easily distinguished from other species of the genus by its thick (≤2.5 mm) resupinate basidiocarps, two types of setae that sometimes are denticulate, and ellipsoid to broadly ellipsoid basidiospores; it causes a distinct white pocket rot of angiosperm wood.

Two species, *Hymenochaete tongbiguanensis* T.X. Zhou & L.Z. Zhao and *H. spinulosetosa* Parmasto, also have thick basidiocarps and denticulate setae. *Hymenochaete tongbiguanensis* can be distinguished from *H. biformisetosa* by its smaller setae (22.5–42 × 3.5–8 µm) and basidiospores (3–4 × 1.5–2 µm; Zhang & Dai 2005), and *H. spinulosetosa* has large, broadly ellipsoid basidiospores (5–6.2 × 3.2–3.7 µm) but only one type of setae (50–75 × 5–7.5 µm; Parmasto 2012).

Six other species of *Hymenochaete*, including *H. denticulata*, also produce denticulate setae, but they differ from *H. biformisetosa* in having thin basidiocarps (<150 µm), shorter setae (40–60 µm), and shorter basidiospores (<5 µm, Léger 1998; Parmasto 2012). *Hymenochaete unicolor* Berk. & M.A. Curtis is similar to *H. biformisetosa* in basidiocarp morphology and anatomical structure with smaller, smooth setae (35–60 × 5–9 µm, Parmasto 2005).

In the phylogenetic tree (Fig. 1), *Hymenochaete biformisetosa* appears to be closely related to *H. denticulata* (bootstrap value = 92%), which also has two types of denticulate setae (Léger 1998; He & Li 2014). The differences between these two species are discussed above. The new species is related also to the

H. cruenta group in the phylogenetic tree (Fig. 1); however, species of this group have red-colored basidiocarps, dendrohyphidia, and cylindrical basidiospores (Léger 1998; Parmasto 2001; Parmasto & Gilbertson 2005).

Of the nine *Hymenochaete* species with denticulate setae now described (Gilbertson & Hemmes 1997; Léger 1998; Zhang & Dai 2005; Parmasto 2012), four are known from China (Zhang & Dai 2005; He & Li 2014).

Key to species of *Hymenochaete* with denticulate setae

1. Basidiocarps <0.15 mm thick 2
1. Basidiocarps up to 1 mm or more 7
2. Well-developed hyphal layer present 3
2. Hyphal layer absent 4
3. Setae 30–40 µm long, with 1–3 teeth ..... ***H. separabilis***
3. Setae 40–70 µm long, with 5–10 teeth ..... ***H. tomentelloidea***
4. Setae with 1–2 teeth ..... ***H. pellicula***
4. Setae with several to many teeth 5
5. Setae of two types ..... ***H. denticulata***
5. Setae uniform 6
6. Cortex present; spores subglobose, 3.7–4.3 µm wide ..... ***H. harpago***
6. Cortex absent; spores ellipsoid to cylindrical, 1.5–2 µm wide ..... ***H. pertenuis***
7. Setae <50 µm long; spores 3–4 × 1.5–2 µm ..... ***H. tongbiguanensis***
7. Setae >50 µm long; spores >4 × 3 µm 8
8. Setae of two types, large setae up to 130 µm long ..... ***H. biformisetosa***
8. Setae uniform, up to 75 µm long ..... ***H. spinulosetosa***

Acknowledgments

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New records of *Dothideomycetes* from Mexico

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Abstract —Ten dothideomycetous species were studied. New records for the Mexican mycobiota include *Heptameria obesa*, *Leptospora rubella*, *Macrovalsaria megalospora*, and *Psiloglonium clavisporum*, and an extended distribution for rarely reported dothideomycetes in Mexico is reported for *Anteaglonium abbreviatum, Astrosphaeriella trochus, Gloniopsis praelonga, Hysterobrevium mori, Oedohysterium insidens*, and *Rhytidhysteron rufulum*. Observations and photographs on macro- and microscopic characters are provided.

Key words— *Ascomycota*, taxonomy, chorology

Introduction

Most fungi with ascostromatic development and bitunicate asci are generally associated with the *Dothideomycetes*, the ascomycete class with the highest number of species (slightly more than 19,000; Kirk et al. 2008). They are found in nature as pathogens, endophytes or epiphytes of living plants, and saprobes that degrade cellulose or other carbohydrates in organic matter, litter, and dung.

Knowledge about ascomycetes in Mexico is still low compared to some European countries where more research has been completed on ascomycete mycological diversity. In Mexico, Medel (2007a) cited 687 ascomycete species, while González & Hanlin (2008) reported 880 species, excluding lichenized ascomycetes. *Pezizales* and *Xylariales* are represented by the greatest number of species records. The first record of a dothideomycetous species for Mexico dates back more than 60 years to *Sphaeria pertusa* Pers. (≡ *Trematosphaeria pertusa* (Pers.) Fuckel) cited by Fries (1851) in Veracruz. Publications on dothideomycetous species of Mexico include Hyde (1992), San Martín (1996), San Martin & Lavin (1999), Checa et al. (2002), Medel (2007b), and Méndez-Mayboca et al. (2010).

Materials & methods

Specimens of *Dothideomycetes* examined included those deposited in the XAL fungal collection as well as collections obtained by S. Chacón & F. Tapia in Veracruz and southeast Mexico during November 2011–September 2012.

Hysterothecia and pseudothecia were examined using a stereoscopic microscope, and microscopic structures were examined using a light microscope. Ascomata were sectioned using a razor blade and mounted in KOH (3% or 5%) and Meltzer solutions as needed. Asci, ascospores, pseudoparaphyses, and peridial thickness were measured.

Species were identified consulting Zogg (1962), Dennis (1981), Sivanesan (1984), Barr (1990a,b), and Boehm et al. (2009). Complete descriptions are provided for species newly recorded for Mexico, and a taxonomic summary and brief notes are presented for the species previously recorded. The recent collections have been deposited in the Herbarium of the Instituto de Ecología A.C. Xalapa (XAL), Veracruz, Mexico.

Taxonomy

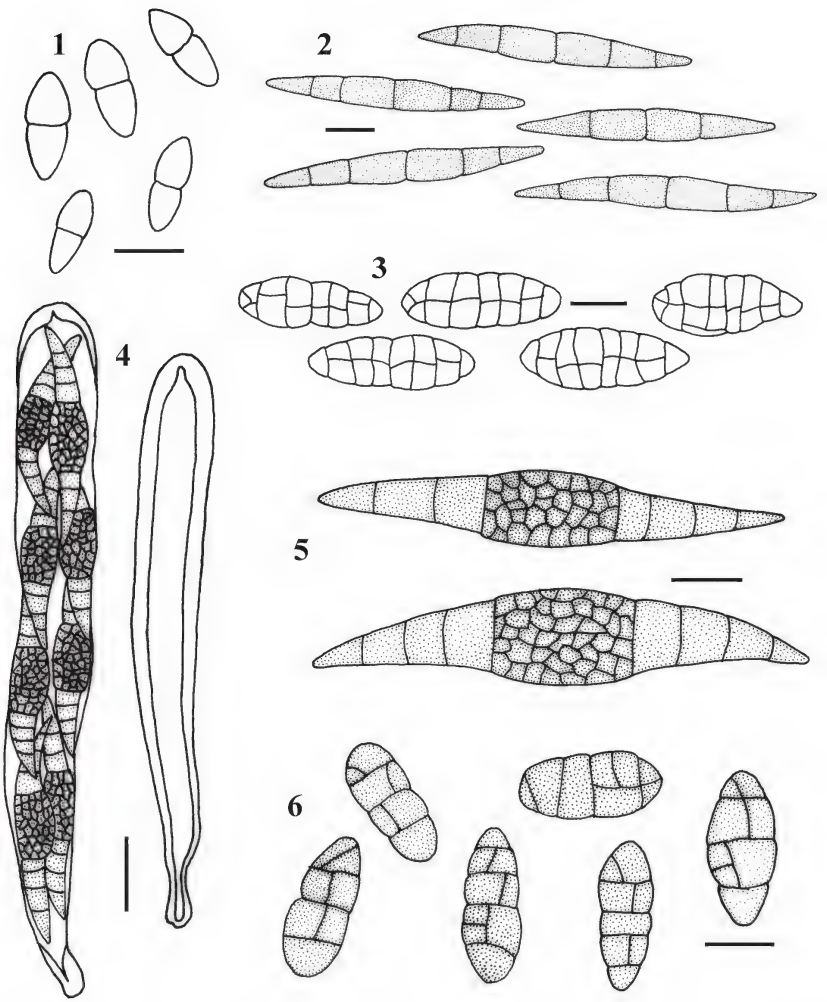
Anteaglonium abbreviatum (Schwein.) Mugambi & Huhndorf, Syst. Biodiv. 7(4): 460 (2009) FIGS. 1, 15

≡ *Glonium abbreviatum* (Schwein.) M.L. Lohman

OBSERVATIONS— *Anteaglonium abbreviatum* is characterized mainly by linear hysterothecia with truncated apices, which allow some blackening on the substrate and spores that are fusiform, bicellular, constrained at the septum, and measure (6.5–)7–7.5(–8) × 2.5–3 µm. *Anteaglonium globosum* Mugambi & Huhndorf (6–7 × 2–3 µm) and *A. parvulum* (W.R. Gerard) Mugambi & Huhndorf (6–8 × 2.5–3 µm) have similarly sized and shaped spores (Boehm et al. 2009) but differ in the form of hysterothecium. In *A. globosum* the hysterothecium is globose (as the name suggests) and roughened, while in *A. parvulum* the hysterothecium is linear but with acuminated apices. Spore sizes for *A. abbreviatum* (as *Glonium abbreviatum*) have been described as measuring 6–8 × 2–5 µm (Lohman 1937), (5–)6–7(–8) × 2–3(–3.5) µm (Zogg 1962), and 7–8 × 3 µm (Romero 1987). *Anteaglonium abbreviatum* is widely distributed and was recently cited by Méndez-Mayboca et al. (2010, as *G. abbreviatum*) from Sonora. It is reported for the State of Veracruz here for the first time.

HABITAT— On dead wood in forest relict of *Fagus grandifolia* var. *mexicana* (Martínez) Little, 850-1900 m asl.

SPECIMENS EXAMINED – **MEXICO. VERACRUZ: Municipality of Emiliano Zapata**, CHAVARRILLO-EL PALMAR ROAD on Monte Oscuro summit, 13 October 2004, S. Chacon 5530; **Municipality of Acatlán**, ACATLÁN VOLCANO upper south part, 11 November 2011, S. Chacón & F. Tapia 6613; **Municipality of Xalapa**, PARQUE NATURA natural protected area, 1 November 2011, S. Chacón & F. Tapia 6585; HOUSE GARDEN on Adolfo Ruiz Cortines Ave., ~200 m from Hospital de Especialidades Médicas, 27 August 2012, S. Chacón & F. Tapia 6849; COLONIA EL OLMO, ex-hacienda Las Ánimas, 27 August 2012, S. Chacón & F. Tapia 6861. **SONORA: Municipality of Álamos**, EL AGUAJE, 14 September 2006, S. Chacón 5764.



Figs 1–6. ***Anteaglonium abbreviatum***, 1: ascospores. ***Astrosphaeriella trochus***, 2: ascospores. ***Gloniopsis praelonga***, 3: ascospores. ***Heptameria obesa***, 4: mature and immature asci. 5: ascospores. ***Hysterobrevium mori***, 6: ascospores. Scale bars: 1= 5 µm; 2–6= 10 µm

Astrosphaeriella trochus (Penz. & Sacc.) D. Hawksw., Bot. J. Linn. Soc. 82: 46 (1981)
Fig. 2, 16

Observations— The studied material is consistent with Hawksworth (1981, except for the spore color, cited by Hawksworth as reddish-brown), and agrees with San Martín & Lavin (1999, who described olivaceous-brown to pale-

brown spores). This is the second record of the species in Mexico and the first for the state of Veracruz. Previously it was recorded for the state of Chiapas by San Martín & Lavin (1999).

Habitat— On stems of *Bambusa* sp. and an undetermined grass in a semi-deciduous forest and a coffee plantation; 460-1300 m asl.

Specimens examined – **MEXICO. Veracruz: Municipality of Actopan**, Km 28 Xalapa-Veracruz highway, near the highest turn to Balastrera, 7 September 1995, S. Chacón 4930; **Municipality of Xico,** near Texolo waterfall, 22 October 1999, S. Chacón 5216; **Municipality of Xalapa**, El Olmo section, southeast Xalapa, 15 August 2012, S. Chacón & F. Tapia 6820; Francisco Javier Clavijero Botanic Garden, Km 2.5 old road Xalapa-Coatepec, 22 August 2012, F. Tapia 2637; INECOL Cloud Forest Sanctuary, km 2.5 old road Xalapa-Coatepec, 26 August 2011, S. Chacón, E. Utrera, F. Tapia & G. Medrano 6293.

Gloniopsis praelonga (Schwein.) Underw. & Earle, Bull. Alabama Agricultural Experiment Station 80: 196 (1897) Fig. 3, 17

Observations— *Gloniopsis praelonga* is distinguished by its spores (18–) 20–30(–31) × 8–12(–13) µm, hyaline, ovoid with obtuse ends, inequilateral, with (5–)6–7 transverse septa and 2–3 longitudinal septa and by presenting linear ellipsoid erumpent to superficial hysterothecia. The studied material is consistent with the descriptions of Zogg (1962), Barr (1990a), and Boehm et al. (2009). Previously recorded for Mexico from the State of Sonora by Méndez-Mayboca et al. (2008), it is recorded here for the first time for the State of Veracruz.

Habitat— On dead branches in a forest relict of *Fagus grandifolia* var. *mexicana* and cloud forest; 1900 m asl.

Specimens examined – **MEXICO. Veracruz: Municipality of Acatlán**, Acatlán volcano upper south, 14 November 2011, Chacón & Tapia 6618; 25 November 2011, Chacón & Tapia 6641; **Municipality of Xalapa**, Francisco Javier Clavijero Botanic Garden, Km 2.5 old road Xalapa-Coatepec, 10 March 1995, F. Tapia 1291; 28 February 1997, F. Tapia 1638; Montevideo section, southeast part of the city, 30 July 2012, F. Tapia 2595; 15 October 2012, S. Chacón 6872; Lázaro Cárdenas Ave. central green area, ~200 m from the State Treasury Office, 22 August 2012, S. Chacón & F. Tapia 6843.

Heptameria obesa (Durieu & Mont.) Sacc., Syll. Fung. 2: 88 (1883) Figs. 4, 5, 18

Pseudothecia subepidermal to suberumpent in the cortex, solitary or densely confluent, subglobose to ovoid with a flat base, papilla conspicuous, completely black, 370–600(–710) µm wide and 380–560(–600) µm high; peridium 110–280 µm thick, composed of thick-walled isodiametric cells, dark brown to black. Asci (120–)130–190 × 17–22 µm, claviform, bitunicate, octosporous, the base shortly stipitate. Cellular pseudoparaphyses. Ascospores (45–) 50–72(–75) × 9–13(–14) µm, fusiform, straight to slightly curved, with 6–9 septa, dictyoseptate or muriform, the average cell is dark-brown to dark-reddish

and from brown pale to hyaline towards the ends with 2–3(–4) transverse septa, slightly constricted at the septum that separates the middle portion; biseriate to occasionally uniseriate in the ascus.

Habitat— On dead wood (*Pseudotsuga* sp.) and dead branches (*Baccharis* sp.), on mesquite vegetation mixed with *Quercus* spp. and *Pseudotsuga* sp. in a *Pinus-Pseudotsuga-Quercus* forest; 2300-2600 m asl.

Specimens examined – **MEXICO. Puebla: Municipality of Salvador el Seco**, Km 62 Perote-Puebla road level with height of the entrance to La Caída, 15 August 1996, S. Chacón 5091. **Veracruz: Municipality of Perote**, Perote-Puebla road 500 m after Perote, turn 10 km to the right, 24 September 2010, S. Chacón & E. Utrera 602.

Observations— *Heptameria obesa* is characterized by the shape of its spores (fusiform, like bull horns) with a dictyoseptate or muriform central part and only the ends with transverse septa. The studied material coincides with the descriptions given by Lucas & Sutton (1971), Barr (1990a), Sierra-López (2006), and Catania & Romero (2010). *Heptameria* has been placed in *Pleosporaceae* by von Arx & Müller (1975) and in *Leptosphaeriaceae* by Barr (1987, 1990a). Kirk et al. (2008) placed it within *Dothideomycetes*, with uncertain familial affinity. *Heptameria obesa* is known from Algeria, Argentina, Spain, USA, France, Portugal, and Taiwan. This is the first record of the species in Mexico.

Hysterobrevium mori (Schwein.) E.W.A. Boehm & C.L. Schoch, Stud. Mycol. 64: 62 (2009) Figs. 6, 19

≡ *Hysterographium mori* (Schwein.) Rehm

Observations— *Hysterobrevium mori* is one of the world's most common species and comprised the species with the second highest number of specimens found in our study. It is distinguished from other *Hysterobrevium* species primarily by its ellipsoid muriform spores of (13–)16–23(–25) × (7–)8–10 µm, with 3–5 transverse septa, as cited by Zogg (1962), Dennis (1981), and Barr (1990a) (all as *Hysterographium mori*) and Boehm et al. (2009) and Lorenzo & Messuti (2009). The species was recently reported from Sonora by Méndez-Mayboca et al. (2010; as *Hysterographium mori*). Here its distribution is extended to the states of Oaxaca, Puebla, Tamaulipas, and Veracruz.

Habitat— On dead branches of *Acacia constricta* A. Gray, *Helietta parvifolia* (A. Gray) Benth, and *Cercidium praecox* (Ruiz & Pav.) Harms, in various types of xerophytic scrub vegetation, tropical deciduous forest, tropical forest, and *Quercus* forest, from 10–1550 m asl.

Specimens examined – **MEXICO. Oaxaca: Municipality of Teotitlán**, Valley of Teotitlán, Km 73, Teotitlán-Cuicatlán road, 25 January 1991, L. Montoya 1949, 1950. **Puebla: Municipality of Zapotitlán Salinas**, Botanic Garden of the Biological Station of UNAM, 16 April 1999, S. Chacón 5129, 5131; 17 April 1999, S. Chacón 5134; 12 June 1999, S. Chacón 5151; 1.5 Km Turn to Santiago Coatepec, Huajuapan Zapotitlán road, 13 December 1997, S. Chacón 5110, 5112; Flores s/n. **Sonora:**

Municipality of Álamos, Huerta Vieja, 12 September 2006, S. Chacón 5736; Palo Injerto, 13 September 2006, S. Chacón 5747; Mesa del Trigo, 14 September 2006, S. Chacón 5769; **Municipality of Fronteras**, La Valdeza, 29 August 2007, S. Chacón 5856; **Municipality of Cumpas**, La Selva, 30 August 2007, S. Chacón 5864. **Tamaulipas: Municipality of Hidalgo**, Km 79 Ciudad Victoria-Linares road, 19 April 1996, S. Chacón 5026, 5028. **Veracruz: Municipality of Xalapa**, Parque Natura protected natural area, 16 August 2011, S. Chacón & E. Utrera 6232; 1 November 2011, S. Chacón & F. Tapia 6584; **Municipality of Actopan**, La Mancha, Biological Station CICOLMA, 14 October 2004, S. Chacón 5536.

Leptospora rubella (Pers.) Rabenh., Klotzschii Herb. Viv. Mycol., Edn 2: no. 532 (1857) Figs. 7, 8, 20

≡ *Ophiobolus rubellus* (Pers.) Sacc.

Pseudothecia subepidermal, gregarious to just a few, pyriform, short necked, sometimes broad and straight at the base resembling a flask, 450 µm high and 300 µm wide, black or sometimes the host with reddish-purple dye. Peridium 35–60 µm thick, formed by sub-globose cells 5–10 µm, periphery cells dark-brown and those towards the inside, hyaline, thick wall. Asci 125–190(–200) × (5–)6–7(–7.5) µm, cylindrical, bitunicate, basal part with a short stipe. Pseudoparaphyses abundant, filiform, hyaline, septate, 1–3 µm thick, anastomosed. Spores 120–180 × 1–1.5 µm, hyaline to yellowish, cylindrical, transversely multiseptate, irregularly spiral within the ascus.

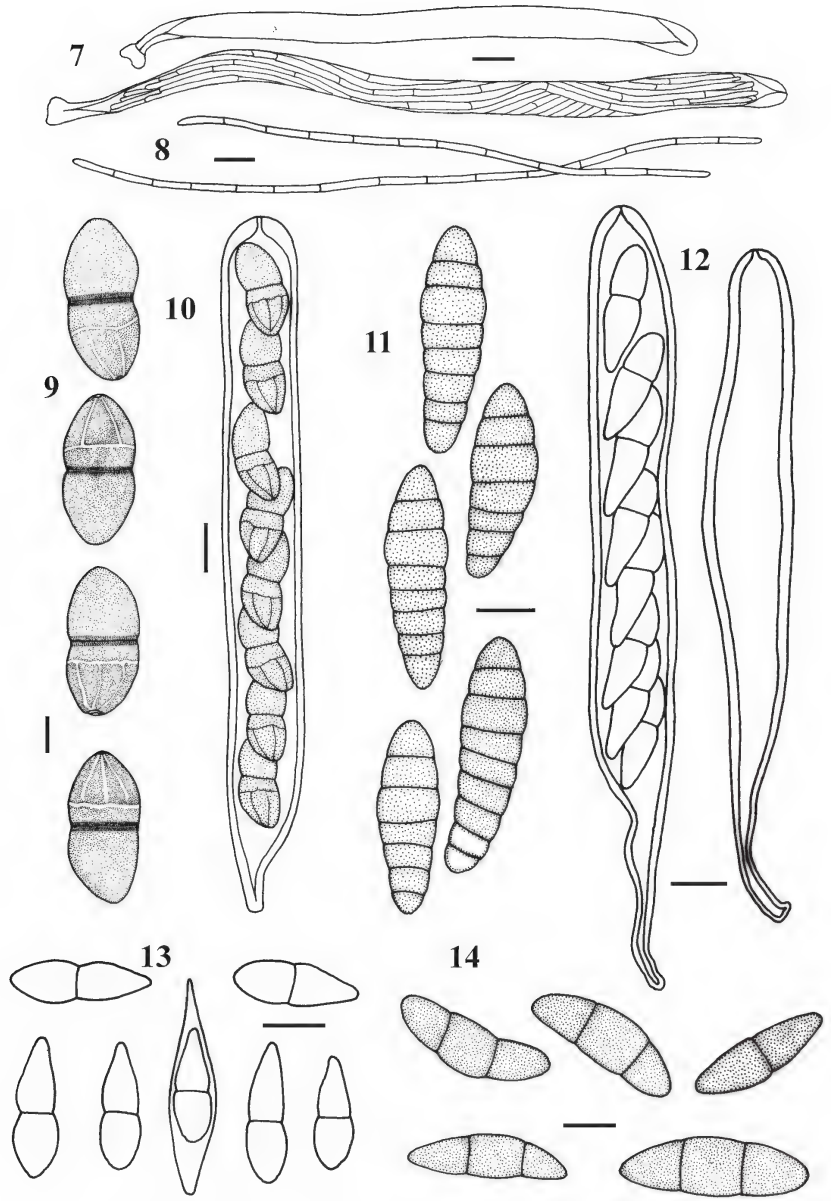
Specimen examined – **MEXICO. Veracruz: Municipality of Xalapa**. Francisco Javier Clavijero Botanic Garden, Km 2.5 old road Xalapa-Coatepec, 2 December 2011, S. Chacón & F. Tapia 6660.

Observations— The main features of this species are reddish-purple spots that present both pseudothecia and the host and the sub-cylindrical spiral spores with abundant septa and spore and ascus measurements that different authors described as very variable. Furthermore, this coincides well with the descriptions of Shoemaker (1976: as *Ophiobolus rubellus*), Walker (1980), Dennis (1970), and Crous et al. (2006). *Leptospora rubella* is known from North America and Europe. This is the first record of the species for Mexico.

Macrovalsaria megalospora (Mont.) Sivan., Trans. Br. Mycol. Soc. 65(3): 400 (1975) Figs. 9, 10, 21

≡ *Macrovasaria leonensis* (Deighton) Petr.

Ascoma solitary or sometimes confluent, unilocular to multilocular, sub-immerse and erumpent, with traces of bark around the ascomata, subglobose-conical, with flat base, 500–1000 µm diam. and 400–750 µm high, ostiolate with a well developed papilla, which contains periphyses, black. Peridium 50–80 µm thick, the outer portion covered by a crusty layer and the inner formed by isodiametric hyaline–dark cells of intricate texture with numerous small dark-brown to nearly black particles, Asci 250–290 × 25–30 µm, cylindrical,



Figs 7–14. ***Leptospora rubella***, 7: immature and mature asci. 8: ascospores. ***Macrovalsaria megalospora***, 9: ascospores. 10: mature ascus. ***Oedohysterium insidens***, 11: ascospores. ***Psiloglonium clavisporum***, 12: mature and immature asci. 13: ascospores. ***Rhytidhysteron rufulum***, 14: ascospores. Scale bars: 7–9, 11–14 = 10 μm; 10 = 20 μm

shortly stipitate, fissitunicate, octosporous and with a clear ocular chamber. Pseudoparaphyses filamentous, septate, anastomosed, ≤2.5µm thick, hyaline. Spores 38–44 × 18–20 µm, ellipsoid, finely verrucose, uniseriate, pale-brown-dark brown, with a central septum strongly constrained and darker than the rest of the spore, one of the ends with helmet-like structure or cover with clear linear halos like striae covering ± ¾ of the total of the spore.

Habitat— On stems of *Cassia fistula* L. in secondary vegetation type, at 20 m asl.

Specimen examined – **MEXICO. Veracruz: Municipality of Cotaxtla**, near the Cotaxtla Experimental field, 26 August 1996, A. Romero s/n.

Observations— Ellipsoid spores with a helmet-like cover with striae toward one end, the presence of a single septum, and the dark-brown color are diagnostic features of this species. *Macrovalsaria leonensis* was treated by Sivanesan (1975) as a synonym of *M. megalospora*; consequently, *Macrovalsaria* remains a monotypic genus. The Mexican material coincides well with the description given by Sivanesan (1975), Hyde (1994), and Wang & Lin (2004). *M. megalospora* is a pantropical species known from Africa, Asia, and South America. This is the first record of the species for Mexico.

Oedohysterium insidens (Schwein.) E.W.A. Boehm & C.L. Schoch, Stud. Mycol. 64: 59 (2009) Figs. 11, 22

≡ *Hysterium insidens* Schwein.

Observations— *Oedohysterium insidens* is easily distinguished from other *Oedohysterium* species by fusiform spores with 6–9 transverse septa and a wider supra-median part 30–40(–45) × 10–11(–12) µm. In contrast, the spore size variability is evident. Zogg (1962) and Boehm et al. (2009) both cite spore sizes of (20–)23–28(–38) × (5–)7–10(–13) µm, while Dennis (1981) cites 21–38 × 6–12 µm and Sivanesan (1984) 20–38 × 6–12 µm. The species was recorded for Sonora by Méndez-Mayboca et al. (2008; as *Hysterium insidens*) with spores 32–45 × 11–14 µm. Here *O. insidens* is cited for the first time for the State of Puebla.

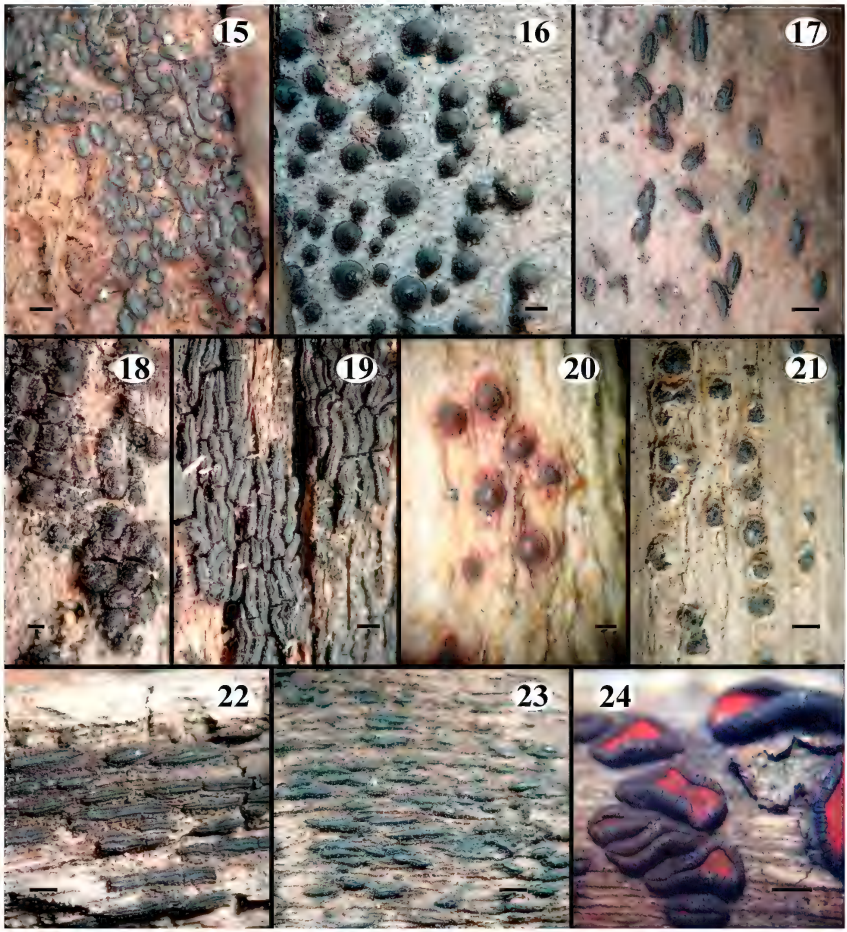
Habitat— On dead wood of *Acacia constricta* in xerophytic vegetation at 1500 m asl.

Specimens examined – **MEXICO. Puebla: Municipality of Zapotitlán Salinas**, Biological Station of the UNAM, 12 June 1999, S. Chacón 5142. **Sonora: Municipality of Fronteras**, La Valdeza, 25 August 2005, S. Chacón 5627.

Psiloglonium clavisporum (Seaver) E. Boehm, C.L. Schoch & Spatafora, Mycol. Res. 113(4): 469 (2009) Figs. 12, 13, 23

≡ *Glonium clavisporum* Seaver

Hysterothecia gregarious, superficial, arranged in parallel, linear to navicular, some slightly curved, ≤3 mm long, faintly striated longitudinally,



Figs 15–24. Pseudothecia and hysterothecia. 15: ***Anteaglonium abbreviatum*** (S. Chacón 5764): scale bar = 1.5 mm. 16: ***Astrosphaeriella trochus*** (F. Tapia 2637): scale bar = 1.2 mm. 17: ***Gloniopsis praelonga*** (S. Chacón 6872): scale bar = 2.0 mm. 18: ***Heptameria obesa*** (S. Chacón 5091): scale bar = 0.5 mm. 19: ***Hysterobrevium mori*** (S. Chacón 5864): scale bar = 0.75 mm. 20: ***Leptospora rubella*** (S. Chacón & F. Tapia 6660): scale bar = 0.4 mm. 21: ***Macrovalsaria megalospora*** (A. Romero s/n): scale bar = 1.8 mm. 22: ***Oedohysterium insidens*** (S. Chacón 5627): scale bar = 1.2 mm. 23: ***Psiloglonium clavisporum*** (S. Chacón 5004-C): scale bar = 2.8 mm. 24: ***Rhytidhysteron rufulum*** (S. Chacón 5859): scale bar = 0.2 mm.

with apices obtuse or acuminate, black, subiculum absent. Peridium carbonaceous. Asci 95–120 × (10–)12–13 µm, cylindrical, short stiped, hyaline to yellowish. Pseudoparaphyses anastomosed, cylindrical 1–2 µm thick, hyaline to olivaceous-yellow. Spores (16–)18–20(–23) × (5.5–)6–6.5(–7) µm, ellipsoid

to obovoid, asymmetric, with one end rounded and one more sub-fusoid, constrained by a central septum, hyaline, uniseriate in the ascus.

Habitat— On decorticated wood in a semideciduous forest, 4 m asl.

Specimen examined – **MEXICO. Veracruz: Municipality of Tampico Alto**, 10 Km before the Llano de Bustos bridge, Tuxpan-Tampico road, 16 April 1996, S. Chacón 5004-C.

Observations— The spore size and the hysterothecial shape characterize the species. Our specimen coincides well with the descriptions of Seaver (1925), Lohman (1937), and Boehm et al. (2009). According to Zogg (1962), *Glonium clavisporum* (≡ *Psiloglonium clavisporum*) is synonymous with *G. lineare* (Fr.) De Not.; however, Boehm et al. (2009) considered them a separate species, describing for *G. lineare* hysterothecia confluent and smaller spores (10–)12–14(–18) × (4–)5–7(–8) µm. Another species that could be confused with *P. clavisporum* is *P. chambianum* (A.L. Guyot) E.W.A. Boehm & C.L. Schoch, which differs by its wider spores (6–)8–9(–10) µm; Zogg 1962; Boehm et al. 2009). *Psiloglonium clavisporum* is known from Africa, India, and Central and North America. Here it is recorded for the first time for the mycobiota of Mexico.

Rhytidhysteron rufulum (Spreng.) Speg., Anales Soc. Ci. Argent. 90: 177 (1921) ["1920"] Figs. 14, 24

≡*Tryblidiella rufula* (Spreng.) Sacc.

Observations— *Rhytidhysteron rufulum* is mainly characterized by discoid apothecia, with the hymen reddish, yellowish, or even yellow-greenish and in the hysterothecial form in young individuals and apothecial form in mature individuals. Spores ellipsoid, 23–35 × 9–14 µm, with 1–3 slightly constricted transverse septa, which coincides with the descriptions of Dennis (1970; as *Tryblidiella rufula*), Samuels & Müller (1979), Kutorga & Hawksworth (1997), and Mendez-Mayboca et al. (2010).

Guzmán (1983) cited *Hysterium angustatum* Alb. & Schwein. from Yucatán and Méndez-Mayboca et al. (2008) cited *H. truncatulum* Cooke & Peck from Sonora; however, our examination of both specimens on which these authors based their descriptions indicated that they are conspecific with *R. rufulum*. Salinas-Salgado et al. (2012) cited *H. angustatum* from Guerrero, which is the first authentic record of this taxon for Mexico, and Méndez-Mayboca et al. (2010) cited *R. rufulum* for Sonora. This paper extends its distribution to the states of Chiapas, Guerrero, Nuevo León, Puebla, Queretaro, Quintana Roo, Tabasco, Tamaulipas, Veracruz, and Yucatán.

Specimens examined – **MEXICO. Chiapas: Municipality of Ocosingo**, near ruins of Bonanpak, 25 April 2012, F. Tapia & S. Chacón 2525; F. Tapia & S. Chacón 2531; **Municipality of Tuxtla Chico**, Rosario Izapa Experimental Field, Km 18

Cacahuatlán-Tapachula road, 14 December 1992, S. Chacón 4633; 15 December 1995, S. Chacón 4974. **Guerrero**: **Municipality of Tololoapa**, Km 40 Iguala-Ciudad Altamirano road, 2 July 1982, G. Rodríguez 234-A. **Nuevo León: Municipality of Santiago**, km 5 west of El Cerrito, 4 October 1980, S. Chacón 53. **Puebla: Municipality of Francisco Z. Mena**, Moctezuma, along the stream on the estate of Don Felipe Chote, 23 June 2012, S. Chacón 6790; **Municipality of Zapotitlán Salinas**, UNAM Biological Station Botanical Garden, 11 August 1998, S. Chacón s/n; 18 September 1998, J. Flores s/n; 16 April 1999, S. Chacón 5128; 17 April 1999, S. Chacón 5135; 12 June 1999, S. Chacón 5141. **Queretaro: Municipality of Pinal de Amoles**, La Cuesta, Km 3 south of Escanelilla, 15 July 1983, G. Rodríguez 1973. **Quintana roo: Municipality of Santa Matilde**, Puerto Morelos to Vallarta road, ~1 Km from the turn to Tulum, 3 November 1981, A. López 1652 (as *Hysterium angustatum* in Guzmán, 1983). **Sonora: Municipality of Álamos**, near Cuzalito, 16 September 2006, S. Chacón 5812; El Cajón, 13 September 2006, S. Chacón 5744; San Pedro, 13 September 2006, S. Chacón 5757; Promontorios, 12 September 2006, S. Chacón 5711, 5730; **Municipality of Cumpas**, La Selva, 27 August 2005, S. Chacón 5692 (as *Hysterium truncatulum* in Méndez-Mayboca et al. 2008); 30 August 2007, S. Chacón 5859. **Tabasco: Municipality of Cárdenas**, La Chontalpa, Ecological Reserve of the Colegio de Posgraduados, Tabasco Campus, Km 21 Cárdenas- Coatzacoalcos road, 24 April 2012, F. Tapia & S. Chacón 2509; **Municipality of Cunduacán**, Huimango, 7 December 1983, R. Solano 27; **Municipality of Nacajuca**, El Sandinal, 8 February 1984, R. Solano 152; **Municipality of Comalcalco**, Oriente 2ND Section, 7 February 1984, R. Solano 119. **Tamaulipas: Municipality of Matamoros**, ~Km 15 Matamoros-Valle Hermoso road, vía El Sendero level with the turn to the village of El Control, 17 May 2003, S. Chacón 5500, 5501; **Municipality of Casas**, Km 68 Ciudad Victoria-Soto de la Marina road, 12 June 1983, G. Rodríguez 1735; Ejido Camotero, near Mante, 17 August 1980, A. Enock s/n. **Veracruz: Municipality of Actopan**, CICOLMA Biological Station, La Mancha, 25 August 1983, A. Sampieri 158; **Municipality of Coacoatzintla**, 1 km after Coacoatzintla, Xalapa-Naolinco road, 6 November 1984, A. Sampieri 1104; **Municipality of Dos Rios**, ~5 km before reaching Chavarrillo near the Tabiquera and the municipal landfill, 3 October 1995, S. Chacón 4941; 7 km before Chavarrillo, Estanzuela-Chavarrillo road, 22 October 1999, S. Chacón 5221, 5222; **Municipality of Emiliano Zapata**, Chavarrillo and Carrizal local road, 15 November 1995, S. Chacon 4962, 4959; **Municipality of Hidalgotitlán**, San Carlos, Zapata and San Carlos road, 23 April 2012, S. Chacón & F. Tapia 6725, 6726; near El Macayal cemetery, 23 April 2012, F. Tapia 2495, 6717, 6719; **Municipality of Jalcomulco**, Xalapa-Huatusco road 1 Km from turn to Jalcomulco, 2 July 1983, S. Chacón 1289; 26 September 1985, S. Chacón 3096; **Municipality of Minatitlán**, Romita 19 Km from Minatitlán, 23 April 2012, F. Tapia 2505; **Municipality of San Andrés Tlalnehuayocan**, near Plan de Sedeño, on the way to San Andres Tlalnehuayocan, 13 October 1995, S. Chacón 4956; near San Antonio Hidalgo, 14 May 2000, D. Jarvio 509; **Municipality of Xico**, near the waterfall, 22 October 1999, S. Chacón 5209, Francisco Javier Clavijero Botanic Garden, km 2.5 old road, Xalapa-Coatepec 11 October 1989, S. Chacon 4217; **Municipality of Xalapa**, Parque Natura, Southeastern Xalapa, 30 August 2011, S. Chacón & F. Tapia 6301; Cerro Macuiltepec Park, Northern Xalapa, 11 October 2011, S. Chacón 6458; 25 October 2011, S. Chacón & F. Tapia 6526. **Yucatan: Municipality of Mérida**, 2 Km before turn to Oncan, Mérida-Tixkokob road, 28 October 1984, S. Chacón 2701.

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Chaetospermum malipoense sp. nov. from southwest China

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Abstract —A new *Chaetospermum* species isolated from root of *Coelogyne leucantha* in Yunnan province of southwest China is described as *C malipoense.* It differs from other *Chaetospermum* spp. by the presence of unique conidiomatal setae.

Key words —*Ascomycota,* endophytic fungi, taxonomy

Introduction

Orchidaceae is a diverse family comprising 20,000–35,000 epiphytic and terrestrial species (Cribb et al. 2003; McCormick et al. 2004). Many studies indicate that mycorrhizal fungi play an important role in the stages of seed germination and growth of orchids (Zettler et al. 2004; Yam & Arditti 2009). Additionally, non-mycorrhizal endophytic fungi associated with orchid plants have been found to serve as potential growth promoters and source of bioactivity substances, but studies on these fungi are very limited (Suarez et al 2006).

The genus *Chaetospermum* was established by Saccardo (1892). Ten species and two varieties have been described based on morphological characteristics, e.g., gelatinous conidiomata, conidia with tubular appendages, and holoblastic sympodial conidiogenesis, Molecular analyses have indicated that *Chaetospermum* taxa should be placed in the basidiomycete order, *Sebacinales* (Rungjindamai et al. 2008). Wells & Bandoni (2001) suggested that members of *Efibulobasidium* (*Sebacinales*) represented teleomorphs of *Chaetospermum*. Kirschner & Oberwinkler (2009) also supported this phylogenetic relationship.

During the investigation of endophytic fungi associated with orchid plants in Yunnan province of southwest China, a novel species of the genus *Chaetospermum* was isolated from the root of *Coelogyne leucantha*. We describe it here as a new species, *Chaetospermum malipoense*.

Materials & methods

Malipo county is located in Wenshan zhuang and Miao minority autonomous prefecture in the southeast of Yunnan province, China, at 22°48′–23°33′N 104°33′–105°18′E. The plant *Coelogyne leucantha* grows epiphytically on the tree trunks in subtropical evergreen forests and flowers in May–June (Flora of China Editorial Board of Chinese Academy of Sciences 1999). In 2010, healthy roots of *C. leucantha* were collected in polyethylene bags, transported to the laboratory and placed in a refrigerator at 4°C. The plant species was identified according to Chen et al. (1999).

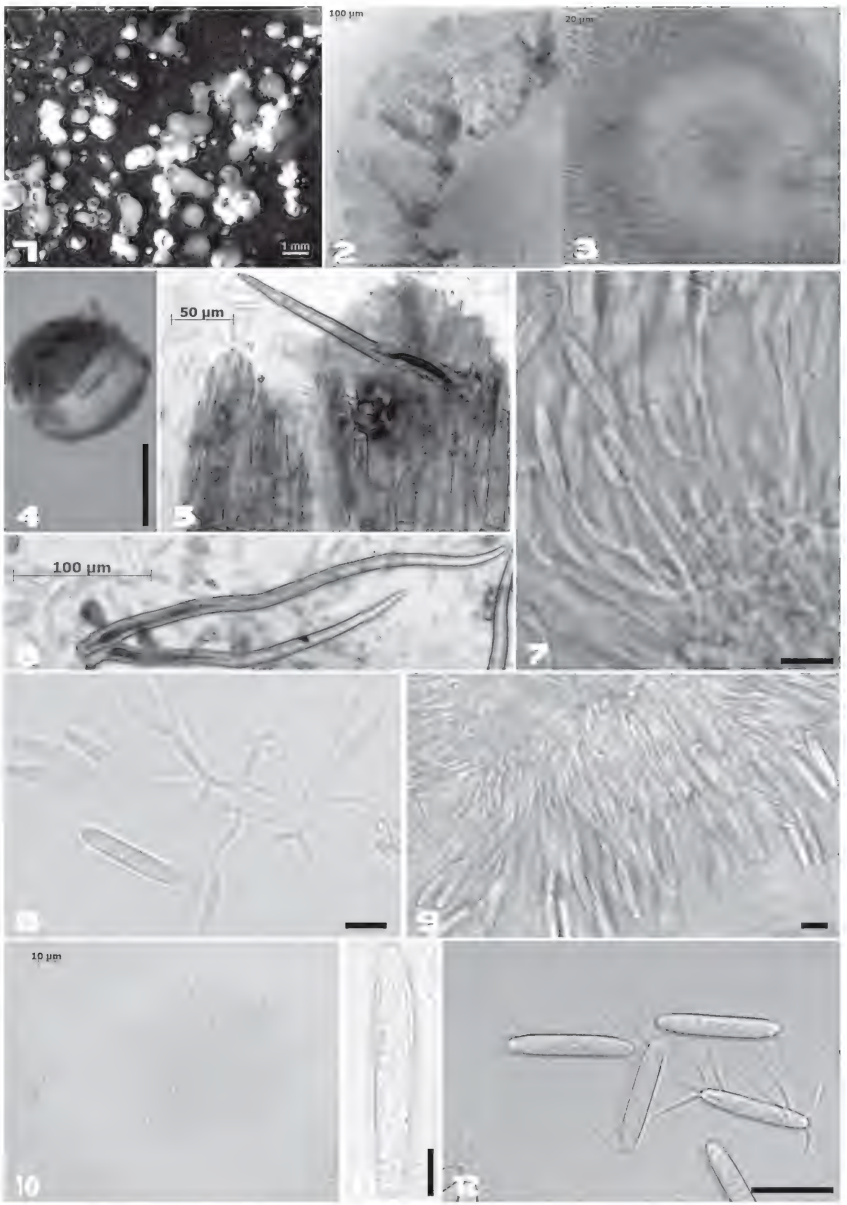
To isolate endophytic fungi, roots of *C. leucantha* washed thoroughly in running tap water were cut into 1 cm segments and surface-sterilized in 70% ethanol for 20 s and 3% $NaClO_2$ for 5 min and rinsed in sterile distilled water 3 times. 3–5 mm segments were separated with the help of a sterile scalpel and were placed on potato dextrose agar (PDA) medium containing 100 µg/ml oxytetracycline and 50 µg/ml streptomycin (Otero et al. 2002). Seven segments were plated per Petri dish, incubated in darkness at 25°C, and checked daily for 4 weeks. Hyphae emerging from segments were transferred to fresh PDA for purity and identification. The pure isolate was morphologically identified and then confirmed by sequence analyses. After extraction of genomic DNA from our isolate, the ITS and 18S rDNA regions were amplified with gene-specific primers (ITS1, ITS4 — White et al. 1990; NS, SL —Inderbitzin et al. 2001) using an iCyclerTM thermal cycler (Mastercycler Gradient). The amplification cycles followed White et al. (1990) and Inderbitzin et al. (2001), respectively. The PCR products were sequenced by Genewiz (Beijing, China) using the same primers as for amplification. Sequences were compared with fungal ITS and 18S rDNA sequences in GenBank using BLAST searches. The fungal isolate was photographed and preserved in the laboratory of Mycology, Biotechnology Center, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences, Beijing; and the culture was accessioned and preserved in China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing. Conidiation structures of *Chaetospermum malipoense* from PDA cultures grown for 20 days at 25°C in darkness were examined, measured, and photographed in a ZEISS Axio Imager A1 (Germany) microscope after mounting in 5% KOH. Measurements were made randomly from 30 cultured specimens and morphology was drawn above tracing paper by free hand and converted into a picture using a scanner.

Taxonomy

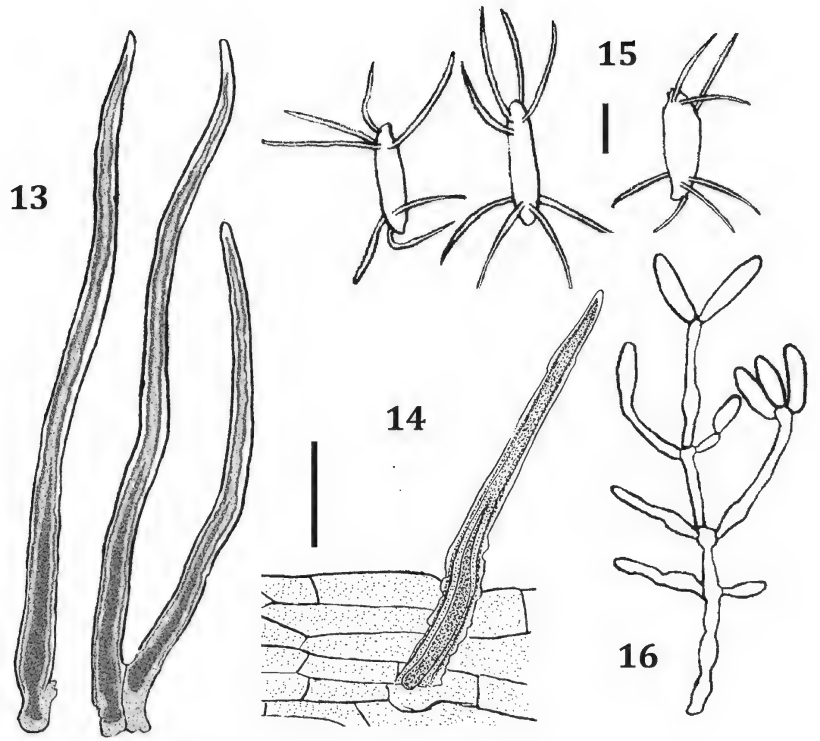
Chaetospermum malipoense X.M. Tan & S.X. Guo, **sp. nov.** Figs 1–16

MycoBank MB 801732

Differs from all other *Chaetospermum* spp. by its aseptate conidiomatal setae and its larger conidia and polar appendages.



Figs 1-12. *Chaetospermum malipoense* (holotype). 1. Habit. 2. Vertical section of conidioma. 3. Conidiophores and conidia. 4. Dehiscent conidiomata. 5. Monothetic conidiomatal setae. 6. Geminate conidiomatal setae. 7–10. Conidiophore branching and conidiogenous cells. 11. Guttulate on the surface of conidia. 12. Conidia with appendages. Scale bars: 2–4 = 250 µm; 5–10 = 10 µm; 11 = 5µm; 12 = 20 µm.



Figs 13-16. *Chaetospermum malipoense* (holotype).
13, 14. Conidiomatal setae. 15. Mature conidia with appendages.
16. Conidiophore branching and conidiogenous cells.
Scale bars: 13, 14 = 50 μm; 15, 16 = 10 μm.

Type: China. Yunnan, Malipo County, in roots of *Coelogyne leucantha* W.W. Sm. (*Orchidaceae*), 17 July 2010, Xiao-ming Tan (**Holotype**, IMPLAD 5880; ex-type culture, CGMCC 6373; GenBank, JQ794486, JQ794487).

Etymology: *malipoense*, referring to the county where the specimen was sampled.

Conidiomata 250–950 μm diameter, opening by a regular split at the base, firstly discrete and globose, ultimately becoming irregular spherical gregarious and confluent, white and gelatinous when moistened. Conidiomatal wall including many layers of non-transparent cells, pseudoparenchymatous at the base, 200–300 μm thick, textura intricata and gelatinous at the upper of conidioma. Setae marginal, 132–362 μm long, 13–23.5 μm wide at the base, light yellow, monothetic to two or three, non-septate at base. Conidiophores arising from the lower half of the conidioma, branched and septate at base,

smooth, firstly fusiform becoming elongate and slender towards the apex with maturity. Conidiogenous cells sympodial, 2–3 in clusters. Conidia mostly cylindrical, smooth, hyaline, (23–)24–32(–37) × (3.5–)4.5–7.5 μm (mean = 27.5 × 5 μm), length/width ratio 5.6:1, abundantly guttulate. appendages subpolar, tubular, unbranched, mostly 3–4 at each end, 14–30 μm long, 1–2 μm wide at the base.

Teleomorph: Unknown.

Comments — We place *C. malipoense* in *Chaetospermum* based on its gelatinous conidiomata, holoblastic sympodial conidiogenesis, and cylindrical non-septate conidia with tubular appendages (Sutton 1980; Nag Raj 1993).

Rajeshkumar et al. (2010) distinguished *C. setosum* from other species by the presence of conidiomatal setae; the 1–2 septa at the base of these setae clearly differ from the aseptate setae of *C. malipoense*. The nearest match for our *C. malipoense* ITS sequence (JQ794486) was an *Efibulobasidium albescens* sequence (AF384860; Wells et al. 2004) with a 95% similarity; however, there are no other *Chaetospermum* ITS sequences in the GenBank database. The nearest match for our *C. malipoense* 18S rDNA sequence (JQ794487) was a *C. camelliae* sequence (EF589729; Rungjindamai et al. 2008) with a 99% similarity. Although *C. camelliae* shares a similar conidial morphology with *C. malipoense,* it can be distinguished by its smaller conidial size (Agnihothrudu 1962).

Acknowledgments

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Chemistry and morphology of *Chrysothrix candelaris* in Poland, with notes on the taxonomy of *C. xanthina*

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Abstract — The chemical and morphological variation of the lichen *Chrysothrix candelaris* s.l. as well as habitat requirements and distribution were studied in Poland. The species was found to be chemically variable, but as the chemotypes do not differ in granule size, habitat preferences, or distribution, they are treated as representing one chemically variable taxon. The size of the granules in Polish specimens is contrary to the previous reports for *C. candalaris,* but the measurements are very similar to those for *C. xanthina*, which differs only in distribution and habitat and is treated as a probable semi-cryptic species.

Key words — secondary chemistry, *Arthoniales*

Introduction

As recently circumscribed, *Chrysothrix candelaris* (L.) J.R. Laundon (Laundon 1981) is an epiphytic (rarely saxicolous) lichen occurring in Europe and characterized by a leprose, yellow, orange yellow, or green yellow thallus consisting of ecorticate granules and the presence of calycin (Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011). According to Kalb (2001) the soredium-like granules (functioning as vegetative propagules) are large, 75–200 µm in diam., but Harris & Ladd (2008) reported them to be much smaller, 50–75 µm in diam.

The species is very similar to *C. xanthina* (Vain.) Kalb (Kalb 2001), which was resurrected from the synonymy of *C. candelaris* s.l., but differs in the production of pinastric acid, granules measuring 20–50 µm in diam. and occurrence in tropical and subtropical world regions and temperate North America (Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011). Although both taxa appear easily distinguishable, some chemically deviating populations exist in the ranges of *C. candelaris* and *C. xanthina,* and these complicate delimitation

of both species. Kalb (2001) noted some European populations containing pinastric acid, therefore chemically similar to *C. xanthina* but morphologically indistinguishable from *C. candelaris*. At first they were considered as probably yet another species (Kalb 2001), but Harris & Ladd (2008) suggested that the material could represent a chemotype of *C. candelaris*. Also within the range of *C. xanthina* is material containing calycin and thus chemically identical to *C. candelaris* but with the granule diameter characteristic for *C. xanthina* (Kalb 2001). Laundon (1981) and Tønsberg (1992) also reported an additional chemotype of *C. candelaris* s.l. from Europe containing both calycin and pinastric acid, but that chemotype was not studied by Kalb (2001) or Harris & Ladd (2008).

Neither Kalb (2001) nor other authors provided studies of *C. candelaris* s.l. chemotypes occurring in Europe in terms of morphological variation, and the problem as to whether the European material represents one chemically variable taxon or a group of species remained unresolved. Although *C. candelaris* s.l. has been rather commonly reported in Poland (Fałtynowicz 2003), its chemistry and morphological variation have never been studied. As we had access to many specimens of this lichen, we studied whether Polish chemotypes differed in granule size, distribution, and habitat preferences to determine if Polish material of *C. candelaris* s.l. represented one taxon. This is particularly important as the species is listed as critically endangered to extinction (Cieśliński et al. 2006).

Material & methods

The material upon which the research was based consisted of 129 samples of *C. candelaris* s.l., all collected in Poland and deposited in KTC, KRAM, LOD, and UGDA. The samples were chemically analyzed with standard procedures of thin layer chromatography (TLC) (Culberson & Kristinsson 1970, Orange et al. 2001). Secondary metabolites were separated in solvent C and compared on each chromatogram with control extracts of calycin, pinastric and vulpinic acids from other lichens.

In order to determine the relation between chemotypes and morphological characters, simple granules were measured(aggregations disintegrating into smaller granules were not considered). For this purpose, granules from 15 specimens were measured (five of each chemotype): 51 granules from chemotype I, 57 from chemotype II, and 37 from chemotype III (chemotype numeration follows Tønsberg 1992; see also results). The measurements were taken in water with addition of ethanol, which reduced the hydrophobic properties of lichen metabolites present in granules and made the granules more easily visible; ethanol was selected, as it did not influence granule size (checked empirically). Because the data were normally distributed and the variance was constant, potential differences in granule sizes between chemotypes were tested by ANOVA F-test and examined by box whisker plots in the STATISTICA v.9.1 (StatSoft Inc. 2010), with abbreviations used in the text: N – total number of granules measured; df – degrees of freedom; p – probability.

All examined localities are mapped according to the ATPOL system in 10 × 10 km squares (Cieśliński & Fałtynowicz 1993; see also Kukwa et al. 2002, 2010, 2012).

Results

Chemotypic variation of *Chrysothrix candelaris* in Poland

Three chemotypes of *C. candelaris* s.l. were found in Poland, the first (I) with pinastric acid as major substance; the second (II) with calycin; and the third (III) containing both calycin and pinastric acid. In chemotypes I and II vulpinic acid was rarely also detected in minor or trace amounts. In our study we found additional variation within chemotype III; one of the substances, calycin or pinastric acid, was more concentrated on TLC plates than the other. That led us to suspect that samples representing chemotype III were only mixed collections of chemotypes I and II. Therefore, we analyzed several parts of thalli occurring on the same piece of substrate to detect if there were chemotypes I and II on the same bark piece, but we always got the same combination of substance concentrations on the TLC plates from all parts of the same specimen. This confirms that chemotype III is not a mechanical "hybrid" formed due to the co-occurrence of individuals containing pinastric acid (chemotype I) and calycin (chemotype II), but a naturally occurring chemotype producing both chemicals.

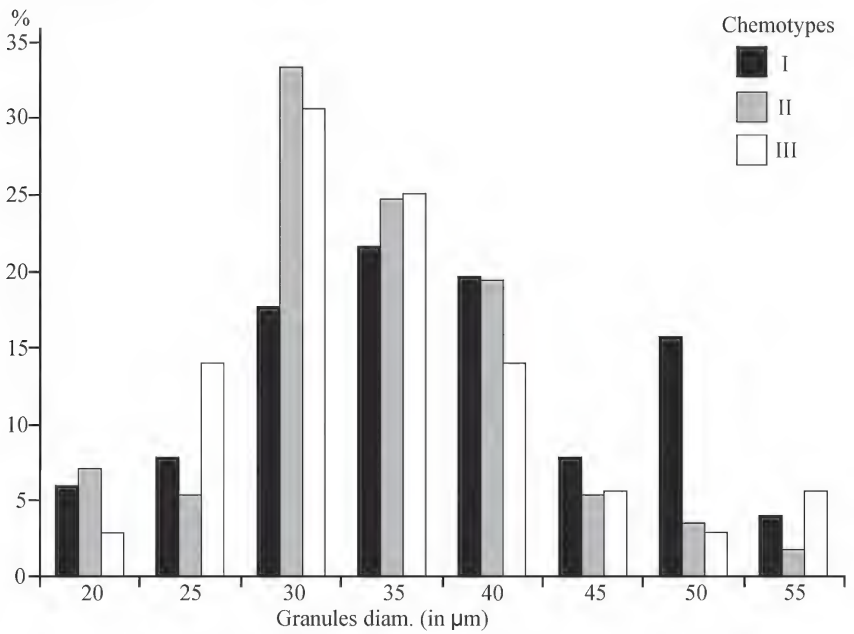

Plate 1. *Chrysothrix candelaris.* Percentage of granule diameter for chemotypes in Poland.

Morphological variation of *Chrysothrix candelaris* in Poland

The granules in 14 samples (including all three chemotypes of *C. candelaris*) were mostly 30–40 µm in diameter with only a few smaller and larger (Plate 1). Only in one sample of chemotype I were several larger granules (55 µm diam.) found. The differences in sizes of granules diameters were not statistically significant between all chemotypes (Anova, F = 2.33, N = 144, df = 2, p = 0.10). Granules in chemotypes II and III were almost of the same average size (34.12 µm versus 34.44 µm), whereas a slightly higher average value was found for chemotype I (37.35 µm) (Plate 2).

Distribution and habitat requirements of *Chrysothrix candelaris* in Poland

The three chemotypes differ in frequencies in Poland: chemotype I is the most frequent (94 samples), whereas II (20 samples) and III (15 samples) are

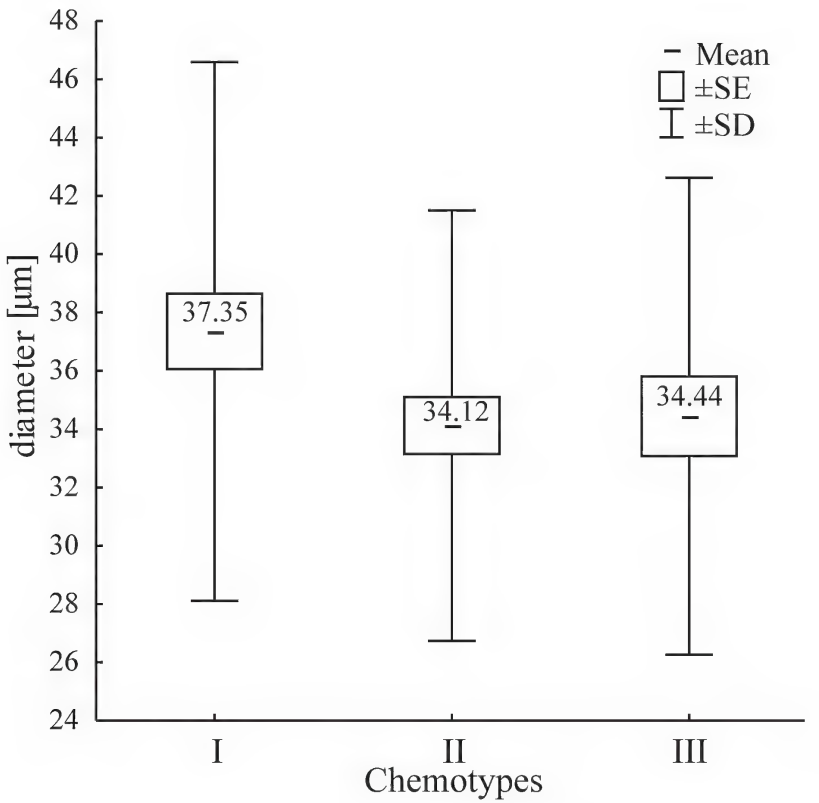

Plate 2. *Chrysothrix candelaris.* Box whisker plot of differences between granule size of chemotypes. Abbreviations: SD – standard deviation, SE – standard error.

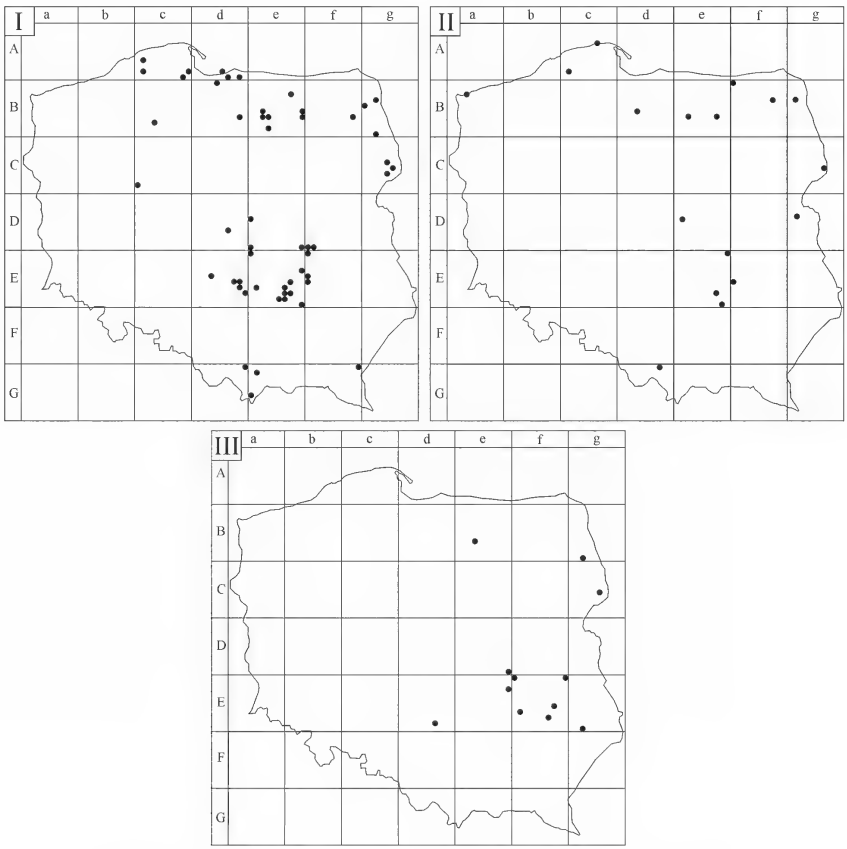

Plate 3. *Chrysothrix candelaris.* Distribution of chemotypes in Poland.

less frequent. Their distributions greatly overlap (Plate 3), and sometimes two different chemotypes were recorded in the same locality (but not growing side-by-side). Only chemotype III is more restricted in distribution, being absent from western Poland.

All chemotypes were most frequently collected on the rough bark of oaks (mostly *Quercus robur*), very often in shady old-growth broad-leafed forests, more rarely on other deciduous trees, conifers (two specimens), and wood (two specimens), and apparently do not differ in habitat preferences.

Discussion

The chemistry of the material referred to as *C. candelaris* in Poland is rather complex and our results are consistent with those of Laundon (1981) and Tønsberg (1992) and also partly of Kalb (2001) and Harris & Ladd (2008).

However, we found an additional, previously unrecognized variation within the third chemotype in which the ratio of the concentration of calycin and pinastric acid varied in different samples. Actually, all specimens can be organized to form a continuum with specimens in which only pinastric acid was found by TLC on one side, those with only calycin detectable on the opposite and samples with both substances in different concentrations in between. Therefore, the boundaries between all chemotypes seem to be less obvious than previously reported. The absence of calycin in chemotype I and pinastric acid in chemotype II can most probably be attributed to the method used for the analyses, and TLC may not be sensitive enough to detect traces of those substances.

When our results of the granule measurements are compared with previous *C. candelaris* treatments, one can, however, see significant discrepancies. Much smaller granules were reported by Tønsberg (1992; (6–)12–25(–30) µm diam.) but much larger by Kalb (2001; 75–200 µm diam.) and Harris & Ladd (2008; 50–75 µm diam.). It is possible that the variation, at least in part, can be due to interaction with environmental factors prevailing in different geographical regions and habitats. To some degree, the earlier reports of large granules were due to the misinterpretation of aggregations as simple granules, as already pointed out by Kukwa & Knudsen (2011).

Kalb (2001) and Harris & Ladd (2008) suggested that *C. candelaris* might consist of more than one species. After studying the material of *C. candelaris* in Poland, we assert that it represents a morphologically uniform entity that is chemically variable. Also the distribution patterns and habitats in Poland do not differ between chemotypes. Under those circumstances, we do not agree that *C. candelaris* is a complex of taxa and that European material containing pinastric acid can represent separate species for which *Lepra citrina* Schaer. is the oldest available name, as was discussed by Harris & Ladd (2008) and Kukwa & Knudsen (2011).

On the other hand, the size of granules in Polish material of *C. candelaris* is consistent with data for *C. xanthina* (Harris & Ladd 2008, Kukwa & Knudsen 2011), which then is hardly distinguishable based on morphology and chemistry; as pointed out above, *C. xanthina* contains pinastric acid, but *C. candelaris* may produce that substance as well. One could regard them as synonymous as proposed by Laundon (1981). However, as *C. xanthina* differs in habitat preferences (e.g., smooth bark and fence-posts exposed to sun and rain, often eutrophicated habitats, burned oak woodlands and chaparral, redwood forests, bishop pine forests vs. *C. candelaris* usually on rough bark, rarely wood and rocks, in humid conditions) and is found in the tropics, subtropics, and temperate North America (vs. *C. candelaris* only in Europe; Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011), *C. candelaris* and *C. xanthina* could be

considered semi-cryptic species, as Vondrák et al. (2009) proposed for some morphologically indistinguishable *Caloplaca* species with different distribution ranges and ecology. We prefer this solution, as there would be a great loss of information if the two taxa are prematurely and unjustifiably united. We therefore propose to keep them separate until more data from all continents, including molecular study, become available.

Selected specimens examined —

Chemotype I. **POLAND. Świętokrzyskie Mts:** Świętokrzyski National Park, Chełmowa Góra, ATPOL Ee–77, on *Abies alba,* 1965, S. Cieśliński (KTC); **Knyszyńska Forest.** Budzisk nature reserve, ATPOL Cg–02, on *Acer platanoides,* 1999, K. Czyzewska et al. (KTC); **Bielska Plain:** Białowieska Forest, forest inspectorate Hajnówka, forest section no. 602D, Myśliszcze range, ATPOL Cg–64, on *Quercus robur,* 1982, S. Cieśliński, Z. Tobolewski (KTC); **Radomska Plain:** Kozienicka Forest, Zagożdżon nature reserve, ATPOL De–99, on *Quercus robur,* 1968, S. Cieśliński (KTC); 0.75 km N of cross roads in Załamanek, ATPOL Df–91, on *Quercus robur,* 1978, A. Anusiewicz (KTC).

Chemotype II. **POLAND. Kraina Wielkich Jezior Mazurskich:** Mokre nature reserve, by Mamry lake, ATPOL Bf–00, on *Fraxinus excelsior,* 1988, S. Cieśliński (KTC); **Szydłowskie Foothills:** 4 km NW of Bogoria, Mostki forest district, forest section no. 48, Ee–98, on *Quercus robur,* 1986, M. Chyb, K. Toborowicz (KTC); **Kozienicka Forest:** Ponty-Dęby nature reserve, ATPOL Ee–09, on *Quercus robur,* 2003, S. Cieśliński (KTC); **Augustowska Plain:** Augustowska Forest, Perkuć, ATPOL Bg–31, on *Quercus robur,* 1986, S. Cieśliński (KTC); **Mazurska Plain:** Kruklanka range, by road from Marksewo to Babięta, ATPOL Be–67, on *Fraxinus excelsior,* 1989, S. Cieśliński (KTC).

Chemotype III. **POLAND. Bielska Plain:** Białowieska Forest, Białowieża forest inspectorate, forest section no. 396C, ATPOL Cg–55, on *Quercus robur,* 1983, S. Cieśliński, Z. Tobolewski (KTC); **Kozienicka Forest:** Ponty-Dęby nature reserve, ATPOL Ee–09, on wood, 2003, S. Cieśliński (KTC); **Radomska Plain:** Kozienicka Forest, Zagożdżon nature reserve, ATPOL De–99, on *Quercus robur,* 1968, S. Cieśliński (KTC).

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Two new species of *Diacheopsis* from China[1]

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Abstract — Two new species from China in the myxomycete genus *Diacheopsis* are described and illustrated. The distinctive characteristics of *Diacheopsis griseobrunnea* sp. nov. are solitary and grayish brown sporocarps, sparse and uniform capillitium, and minutely warted spores; those of *Diacheopsis gigantospora* sp. nov. are large spores marked with long spines, a capillitium that forms a very loose network, and wide tubular threads. Differences between the new species and closely related taxa are discussed. Type specimens are deposited in the Microbial Cultures Center of Nanjing Normal University.

Key words —morphology, taxonomy

Introduction

The genus *Diacheopsis* (*Stemonitaceae*, *Stemonitida*) was established by Meylan in 1930 with *D. metallica* Meyl. as the type species (Kirk et al. 2008; Martin & Alexopoulos 1969). Sixteen species are currently known for the genus (Hernández-Crespo & Lado 2013), but only one species, *D. mitchellii*, has been collected from China (Yamamoto et al. 2002).

Among our examinations of a number of myxomycetes obtained from moist chamber cultures of bark samples from living trees, two specimens were found that appeared to fit the generic concept of *Diacheopsis*, in that they had sessile fruiting bodies and were limeless, non-columellate, and characterized by an iridescent peridium. As they also differed from any known species of *Diacheopsis*, they are described here as two new species.

Materials & methods

Moist chamber culture: In the laboratory, the bark samples from living trees were placed in artificial moist chambers. These chambers consisted of Petri dishes (9 cm in diam.) lined with moistened filter paper as described by Stephenson (1989).

[1] Shu-Zhen Yan and Ming-Quan Guo contributed equally to this paper.

The samples were moistened with distilled water, and excess water was poured off after approximately 24 h. The cultures were maintained under indirect natural light at room temperatures (23–25 °C). To maintain a moist environment, distilled water was added to the cultures when required. The cultures were examined under the stereomicroscope every 2 days. After 1–2 days when the myxomycete fruiting bodies were fully mature, they were removed from the moist chambers, air-dried, and placed into a small plastic specimen box for permanent preservation. All specimens examined are deposited in the Microbial Cultures Center of Nanjing Normal University (MCCNNU).

Morphological observations: The morphology was observed using a stereomicroscope (Jiangnan JSZ6, Nanjing, China), optical microscope (Nikon YS2–H, China and Zeiss Axio Imager A1, Göttingen, Germany), and scanning electron microscope (JEOL, JSM-5610LV and JSM-5900, Tokyo, Japan). For light microscopic observations, the preparation of slide mounts followed Martin & Alexopoulos (1969). For SEM study, the air-dried specimens with entire and broken sporocarps were attached to double-sided adhesive tape on sample holders of the scanning electron microscope, and coated with platinum-palladium using a JEOL JFC-1600 Auto Fine Coater.

Taxonomy

Diacheopsis griseobrunnea Shuang L. Chen, Shu Z. Yan & M.Q. Guo, **sp. nov.**

MycoBank MB 805456 Plate 1

Differs from *Diacheopsis depressa* by its solitary globose sporocarps, sparse capillitium not forming a network, and smaller spores.

Type: China, Guangxi: Shangsi County, Pingguang Forest Center, alt. 250 m, on bark of living *Pinus massoniana* Lamb. in moist chamber culture, collected 12 Jan. 2005, cultured 5 May 2005, harvested 22 May 2005, S.-Z. Yan & S.-L. Chen (**Holotype**, MCCNNU00151).

Etymology: Latin *griseus* (gray) and *brunneus* (brown), referring to the grayish brown sporocarps.

Fruiting bodies sessile and sporocarpous. Sporocarps solitary, grayish brown, iridescent with purple and metal reflections, globose or subglobose, the base constricted, 0.3–0.6 mm diam. Hypothallus dark brown, round, small, thin, membranous. Peridium persistent, thin, membranous, outer surface roughened with some irregular wrinkles. Columella absent. Capillitium sparse, connected to the base and the peridium, a few branched, threads slender, uniform, 1–1.5 μm in diam., smooth, subhyaline by transmitted light. Spore dark brown in mass, pale violaceous brown by transmitted light, densely marked with minute warts, globose, 6.5–8.0 μm diam. Plasmodium not observed.

Comments: *Diacheopsis griseobrunnea* is similar to *D. depressa* K.S. Thind & T.N. Lakh., *D. mitchellii* Nann.-Bremek. & Y. Yamam., *D. rigidifila* S.L. Stephenson & Nann.-Bremek., and *D. synspora* Nann.-Bremek. & Y. Yamam. in the nature of the capillitium which forms a few branches but not a network. However, *D. depressa* differs by its globose to subglobose to slightly elongated

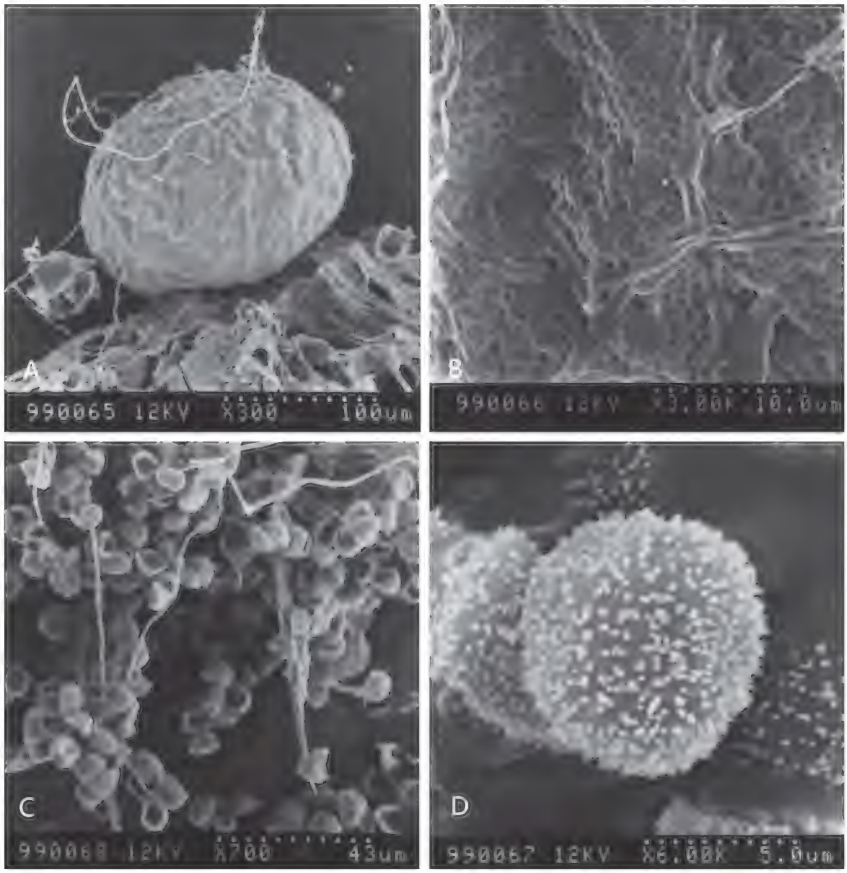

Plate 1. *Diacheopsis griseobrunnea*: A: Sporocarp; B. Portion of the outer peridium; C: Capillitium and spores; D: Spore.

angular sporocarps, its abundant capillitium, and its larger spores (9–12 µm diam.; Thind & Lakhanpal 1968); *D. mitchellii* has larger spores (18–23 µm diam.; Nannenga-Bremekamp & Yamamoto 1983); *D. rigidifila* has a capillitium with more or less fusiform thickenings in the central part (Stephenson & Nannenga-Bremekamp 1990); and *D. synspora* has spores united in stable clusters of 5–7 (Nannenga-Bremekamp & Yamamoto 1986).

Diacheopsis gigantospora Shuang L. Chen, M.Q. Guo & Shu Z. Yan, **sp. nov.** Plate 2
MycoBank MB 805455

Differs from *Diacheopsis mitchellii* by its very loose capillitial network, wider tubular capillitial threads, and larger spores.

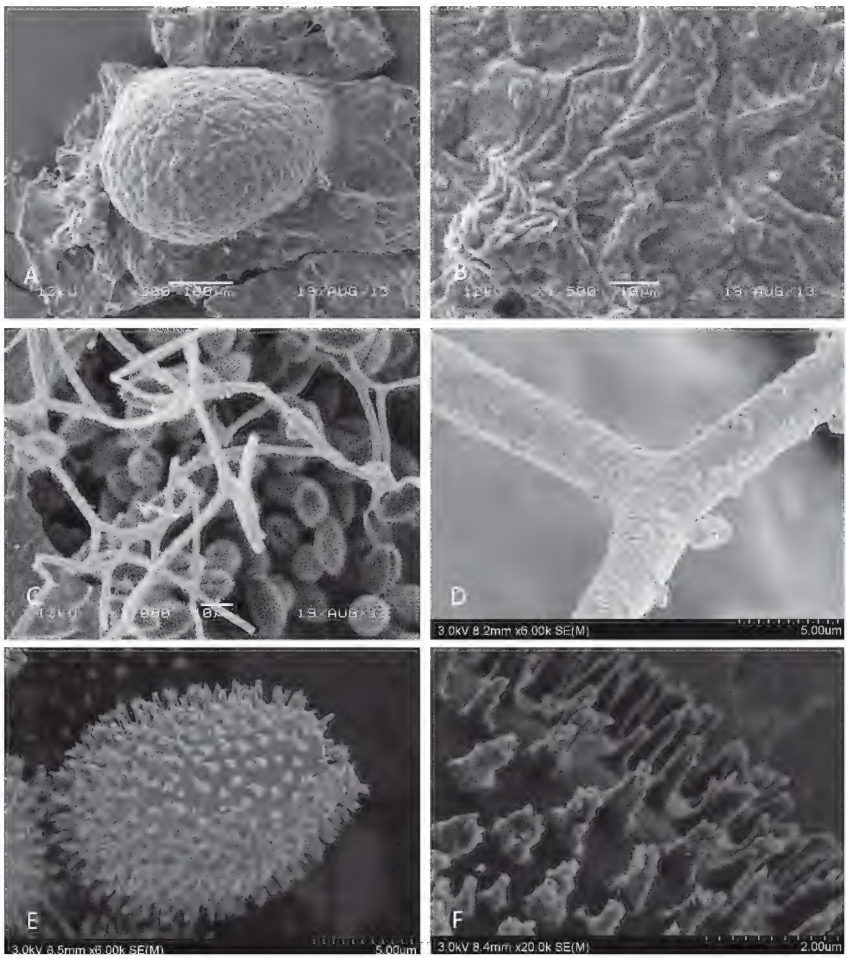

Plate 2. *Diacheopsis gigantospora*: A: Sporocarp; B. Portion of outer periduium; C: Portion of the capillitium net; D: Branched part of the capillitium; E: Spore; F: Part of a spore.

Type: China, Yunnan: Pingbian County, Dawei Mountain National Natural Reserve, alt. 1677 m, on bark of living *Cryptomeria japonica* (Thunb. ex L. f.) D. Don. in moist chamber culture, collected 23 Nov. 2012, cultured 24 Feb. 2013, observed 29 Mar. 2013, harvested 19 Apr. 2013, M.-Q. Guo & S.-L. Chen (**Holotype**, MCCNNU00706).

Etymology: Latin "*gigant-*" (gigantic) and "*sporus*" (spored), referring to the large spores.

Fruiting bodies sessile and sporocarpous. Sporocarps solitary, semi-globose to elongated, occasionally pulvinate, base wide, 0.1–0.2 mm in height, 0.3–0.5 mm in width, up to 0.7 mm in length. Sporocarps black at first, becoming dark

brown to dull black, iridescent with blue and purple reflections when maturing. Hypothallus dark brown, small, thin, membranous. Peridium persistent, thin, membranous, the outer surface mostly roughened but without any distinct ornamentation, the inner surface smooth. Columella absent. Capillitium sparsely branched and with few interconnections, forming a very loose network, threads white or pallid but limeless, straight, tubular, 2–5 μm in diam. and sometimes expanded at the junctions and marked with a few bead-like thickenings on the larger branch. Spores black in mass, dark brown to black brown by transmitted light, obviously marked with evenly-distributed spines up to over 1 μm long, globose, (17.5–)20.6–26.2(–28) μm diam. including spines. Plasmodium black.

Comments: *Diacheopsis* species having large spores include *D. insessa* (G. Lister) Ing (14–19 μm; Martin & Alexopoulos 1969), *D. kowalskii* Mar. Mey. & Poulain (15–18.5 μm; Meyer & Poulain 1998), and *D. mitchellii* (18–23 μm; Nannenga-Bremekamp & Yamamoto 1983). However, the spore sizes in these three species are smaller than those in *D. gigantospora*. In addition, *D. insessa* differs by its densely clustered sporocarps (sometimes with weak stalks) and a purplish capillitium; *D. kowalskii* has gregarious and pulvinate sporocarps, a bicolored capillitium, and flattened threads bearing darker axillary expansions; and *D. mitchellii* has sporocarps in small groups, capillitium of simple or sparsely dichotomous threads ca. 0.5 μm diam. and not forming a network, and spores densely covered with evenly-distributed nail-headed warts or blunt spines.

Several other species of *Diacheopsis* have wide capillitium threads, including *D. kowalskii*, *D. nannengae* G. Moreno et al., *D. pauxilla* Mar. Mey. & Poulain, *D. reticulospora* Mar. Mey. & Poulain, *D. serpula* Kowalski, and *D. synspora*. From *D. gigantospora*, *D. nannengae* differs by its larger sporocarps, brown capillitium, and smaller spores (Moreno et al. 1989); *D. pauxilla* by its gregarious and greater sporocarps, and smaller spores (Meyer & Poulain 1998); *D. reticulospora* by its plasmodiocarpous sporocarps, brown capillitium, and spores densely and irregularly reticulated (Meyer & Poulain 1990); *D. serpula* by its plasmodiocarpous sporocarps, purple-brown threads, and smaller spores (Meyer & Poulain 1998); and *D. synspora* by its gregarious and larger sporocarps, dichotomously branched capillitial threads and spores united in stable clusters of 5–7 (Nannenga-Bremekamp & Yamamoto 1986).

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Australohydnum dregeanum new to Italy

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Abstract — We report on the first finding in Italy (and the third in Europe) of *Australohydnum dregeanum*. A detailed description of the macro- and micromorphological features, the ecological data, and some taxonomic remarks concerning this taxon are provided. The bluish tint in the centre of the hymenophoral surface and the non-cystidiform aspect of the marginal hyphae are previously undescribed characters.

Key words — *Corticiaceae*, *Phanerochaetaceae*, wood-inhabiting fungi, urban biodiversity

Introduction

Wood decay fungi are widely distributed in forest ecosystems of Italy. Their importance is highlighted by the fundamental role they play in the decomposition of woody debris (Saitta et al. 2011) and by the high level of diversity reported from broad-leaved and conifer woods (Venturella et al. 2011). Although in recent years the mycological exploration in forest ecosystems of Italy has definitely increased, it is still possible to find infrequent and/or rare lignicolous species growing as saprotrophs or parasites on fallen trunks, branches, stumps, etc. (Saitta & Melo 2012). Such is the case with *Australohydnum dregeanum*, recently collected and identified as new to Italy (a third record from Europe).

Three species are included in *Australohydnum* Jülich (*Phanerochaetaceae*): *A. castaneum*, *A. dregeanum*, and *A. griseofuscescens*; we follow here the synonymy outlined by Melo & Hjortstam (2002), which treats *A. dregeanum* and *A. griseofuscescens* as conspecific.

Materials & methods

The basidiomata were collected inside the "Parco della Favorita", belonging to the Natural Reserve of "Monte Pellegrino", a wide green area within the city of Palermo

(Sicily). The macroscopic identification was carried out on fresh basidiomata while the microscopic features were observed under a Leica microscope DMLB on dried specimens using a 0.3% KOH solution and cotton blue in lactic acid. The spore measurements were based on 50 observations carried out on fresh and dried basidiomata. The nomenclature follows MycoBank (http://www.mycobank.org), and the description of *A. dregeanum* is based on the authors' observation of the collected basidiomata. The specimens (SAF 001, SAF 002) are kept in the fungal dried reference collection of the mycological herbarium (under establishment) of the new Department of Agricultural and Forest Sciences (activated by the University of Palermo on 2014).

Taxonomy

Australohydnum dregeanum (Berk.) Hjortstam & Ryvarden,
Syn. Fung. 4: 61. 1990. Pl. 1

≡ *Corticium dregeanum* Berk., London J. Bot. 5: 3. 1846.

≡ *Lopharia dregeana* (Berk.) P.H.B. Talbot, Bothalia 6: 57. 1951.

= *Hydnum griseofuscescens* Reichardt, Verh. Zool.-Bot. Ges. Wien 16: 374. 1866.

≡ *Austrohydnum griseofuscecens* (Reichardt) Jülich, Persoonia 10: 138. 1978.

= *Irpex vellereus* Berk. & Broome, J. Linn. Soc., Bot. 14: 61. 1873.

≡ *Oxyporus vellereus* (Berk. & Broome) A. Roy & A.B. De, J. Mycopathol. Res. 36: 41. 1998.

= *Irpex purpureus* Yasuda ex Lloyd, Mycological Notes 50: 715. 1917.

Basidiomata annual, resupinate to effuse-reflexed, forming patches up to 5 cm wide, and laterally confluent and effused ≤15 cm, cracked when dried. The hymenial surface is bluish in the center when fresh, gradually lilac to the margin; brownish when dried, with some areas soft lilac; more or less warted, warts ≤2.5 mm long, differently anastomosed. Margin evident, sterile, distinctly white, slightly tomentose ≤4 mm broad. The basidiomata can be easily separated from the substrate only when dried. Flesh thin, white.

Hyphal system pseudodimitic. Hyphae simple septate. Hymenial hyphae prevalently thin-walled. Subicular hyphae thick-walled, 4–5.5 µm wide. Marginal hyphae 4–5 µm wide, hyaline, more or less thick-walled, frequently branched, sometimes encrusted at the apex. Basidia hyaline, (22–)25–35(–40) µm long, sinuous-clavate, simple septate at the base, with sterigmata 3.5–4.5 µm long. Spores (4.5–)4.8–5.3(–5.8) 2.5–2.8(–3.2) µm, ellipsoid, hyaline, smooth, thin-walled, inamyloid, cyanophilous. Skeletocystidia very abundant, cylindrical ≤125 µm long and 4.5–6.5(–7) wide, apices obtuse, more or less encrusted, originating from pseudoskeletal hyphae and rarely projecting beyond the hymenium ≤15 µm, incrusted part 25–45 µm.

Specimens examined: **ITALY, Sicily**, Palermo, Parco della Favorita, 38.15457°N 13.34628°E, 44 m a.s.l., Mediterranean maquis with scattered plants of *Eucalyptus sideroxylon* A. Cunn. ex Woolls, on fallen trunk of *E. sideroxylon*, 4 Apr 2012, coll. A. Saitta (SAF 001); 5 Oct 2012, coll. A. Saitta (SAF 002).

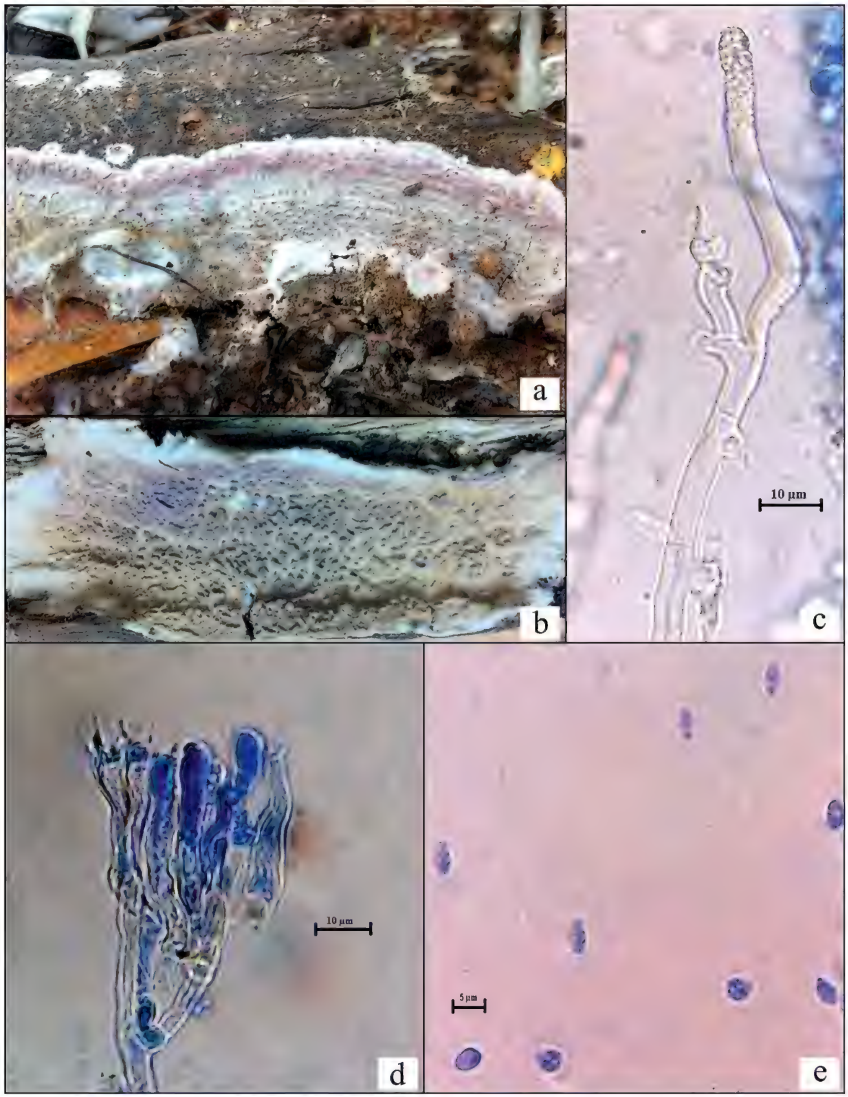

Plate 1. *Australohydnum dregeanum* (SAF 002). a) Fresh basidioma; b) Dried basidioma; c) Skeletocystidium; d) Basidia; e) Spores.

Discussion

Australohydnum dregeanum was collected on fallen trunk of *E. sideroxylon* in the clearings of a Mediteranean maquis. The vegetation is also characterized

by scattered plants and shrubs of *Quercus ilex* L., *Ulmus minor* Mill., and *Pistacia lentiscus* L. mixed with conifers, *Cedrus atlantica* (Endl.) Manetti ex Carrière, *C. deodara* (Roxb. ex D. Don) G. Don, and *Cupressus sempervirens* L.

Eucalyptus sideroxylon was introduced in reforestations approximately 60 years ago, when the local Department of Forestry began an intense planting of exotic species on the Sicilian territory. *Eucalyptus* wood is a new substrate, and the Mediterranean maquis is a new habitat for *A. dregeanum* in Europe. Melo & Hjortstam (2002) reported *A. dreageanum* on fallen branches of *Olea europaea* L. var. *europaea* in a holm oak forest (*Quercus rotundifolia* Lam.) with a small abandoned olive grove and lusitanic oaks (*Q. faginea* Lam.).

Owing to the presence of abundant encrusted skeletocystidia and simple septate generative hyphae, *A. dregeanum* is rather easily identified. The morphological features of the *A. dregeanum* collected in Sicily are similar to those reported by Melo & Hjortstam (2002) for specimens collected in Portugal. The main difference in the features of the Sicilian specimens are the bluish tint in the centre of hymenophoral surface (not reported in any previous description) and the non-cystidiform aspect of the marginal hyphae. The bluish tint in the centre of hymenophoral surface is clearly visible in the fresh specimens.

The genus *Australohydnum* in Europe includes only one taxon, *A. dregeanum*, which was collected in Portugal by Melo & Hjortstam (2002) as new to Europe. The species is also known from Australia (Reichardt 1866, as *Hydnum griseofuscescens*; Reid 1956, as *Irpex vellereus*), New Zealand (Buchanan & Ryvarden 2000), Japan (Lloyd 1917, as *Irpex purpureus*), South Korea (Lim et al. 2005), Sri Lanka (Berkeley & Broome 1873, as *Corticium dregeanum*), India (De 1998, as *Oxyporus vellereus*; Tiwari et al. 2010], South Africa (Berkeley 1846, as *Corticium dregeanum*; Talbot 1951, as *Lopharia dregeana*), Israel (Tura et al. 2010), and Russia (Zmitrovich et al. 2006).

Lim & Jungh (2003) described a new species, *Irpex hydnoides* Y.W. Lim & H.S. Jung, microscopically quite similar to *A. dregeanum* but which has a different hymenophoral configuration. They analyses did not place *Irpex vellereus* within the *I. hydnoides–I. lacteus* clade but grouped it instead (with 100% support) with a sequence of *A. dregeanum*, supporting the synonymy of *A. dregeanum* and *I. vellereus*. Their *A. dregeanum–I. vellereus* clade was sister to the group that included members of the genus *Phanerochaete*.

The presence of *A. dregeanum* in Italy is noteworthy, considering its fragmented European distribution. This new finding also supports Sicily as a "hot spot" of biodiversity for aphyllophoroid fungi (Saitta et al. 2011).

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Anamorphic fungi of the Atlantic Forest of southern Bahia: new records and *Dactylaria pseudomanifesta* sp. nov.[§]

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Abstract — A new species, *Dactylaria pseudomanifesta*, and nine other species newly recorded from Brazil (*Beltraniella fertilis*, *Chaetosphaeria innumera*, *Dictyosporium oblongum*, *Idriella acerosa*, *Inesiosporium longispirale*, *Pseudobeltrania macrospora*, *Scolecobasidium tropicum*, *Sporidesmium coffeicola*, and *Triscelophorus curviramifer*) are presented. The specimens were recovered from the litterfall of *Parinari alvimii*, *Manilkara maxima*, and *Harleyodendron unifoliolatum*, native trees of the Atlantic Forest in Bahia State, Brazil.

Key words — conidial fungi, taxonomy, *Chrysobalanaceae*, *Sapotaceae*, *Fabaceae*

Introduction

The Atlantic Forest is the third largest biome in Brazil (after the Amazon and Cerrado) and the biome of richest biodiversity on the planet (Conservação Internacional do Brasil 2003). It is estimated that this biome contains about 20,000 plant species (of which 8,000 are endemic), accounting for 2.7% of the total number of plants of the world (Myers et al. 2000). Taxonomic studies on decomposer fungi in the Atlantic Forest are necessary, especially because

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it is a biome with a significant biologic diversity which presents a extremely high level of endemism, more strong in the south of Bahia State (Conservação Internacional do Brasil et al. 2000).

New studies on anamorphic fungi in the Atlantic Forest in Brazil have expanded the knowledge of the geographical distribution of species and diversity of fungi present in this biome (Castañeda-Ruiz et al. 2001, 2003; Calduch et al. 2002; Gusmão et al. 2005, 2008; Cruz et al. 2007; Marques et al. 2008; Barbosa et al. 2008, 2009; Magalhães et al. 2011) but not as much in southern Bahia.

Here we present records of conidial fungi isolated from the fallen litter of the Atlantic Forest of south Bahia, including a new species, *Dactylaria pseudomanifesta*, and nine other taxa not previously reported from Brazil.

Materials & methods

The study was done in three conservation units of the biome Atlantic Forest of southern Bahia, Brazil: (1) Ecoparque, in the municipality of Una; (2) Parque Estadual Serra do Conduru (PESC), in the municipalities of Uruçuca, Itacaré and Ilhéus; and (3) Reserva Capitão, in the municipality of Itacaré.

Three specimens of three representative endemic plant species well distributed in the Atlantic Forest of southern Bahia were identified in the field: *Parinari alvimii* Prance (Oiti cumbuca; *Chrysobalanaceae*); *Manilkara maxima* T. D. Penn. (Massaranduba; *Sapotaceae*), and *Harleyodendron unifoliolatum* Cowan (*Fabaceae*). Surveys were conducted on four occasions during the period from December 2007 to July 2008. Plants were identified, georeferenced, and marked.

A 50 × 50 cm square (0.25 m^2) was thrown over the litter of these trees and one leaf was randomly collected at a time, totaling ten leaves/plant under different stages of decomposition. In the Laboratory of Fungal Diversity of Centro de Pesquisa do Cacau (CEPEC) – Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), samples were washed for one hour in running water and incubated in moist chambers (Castañeda-Ruiz et al. 2006).

After 72 hours of incubation the material was observed under the stereomicroscope and the light microscope and periodically reviewed for 30 days. All microfungi specimens were studied in a semi-permanent mounting medium (PVL resin + polyvinyl alcohol lactophenol; Trappe & Schenck 1982) and identified. Colonized leaves and semi-permanent mounts were converted into exsiccates and deposited in CEPEC herbarium.

Taxonomy

Dactylaria pseudomanifesta J.L. Bezerra & D.M.A. Magalh., **sp. nov.** PLATE 1

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Differs from *Dactylaria manifesta* by its larger brown conidia.

TYPE: Brazil. Bahia: municipality of Una, Ecoparque, 15°10′01.7″S 39°03′14.2″W, on decomposing leaves of *Manilkara maxima*, 10/5/2008, D.M.A. Magalhães s.n. (CEPEC 1459).

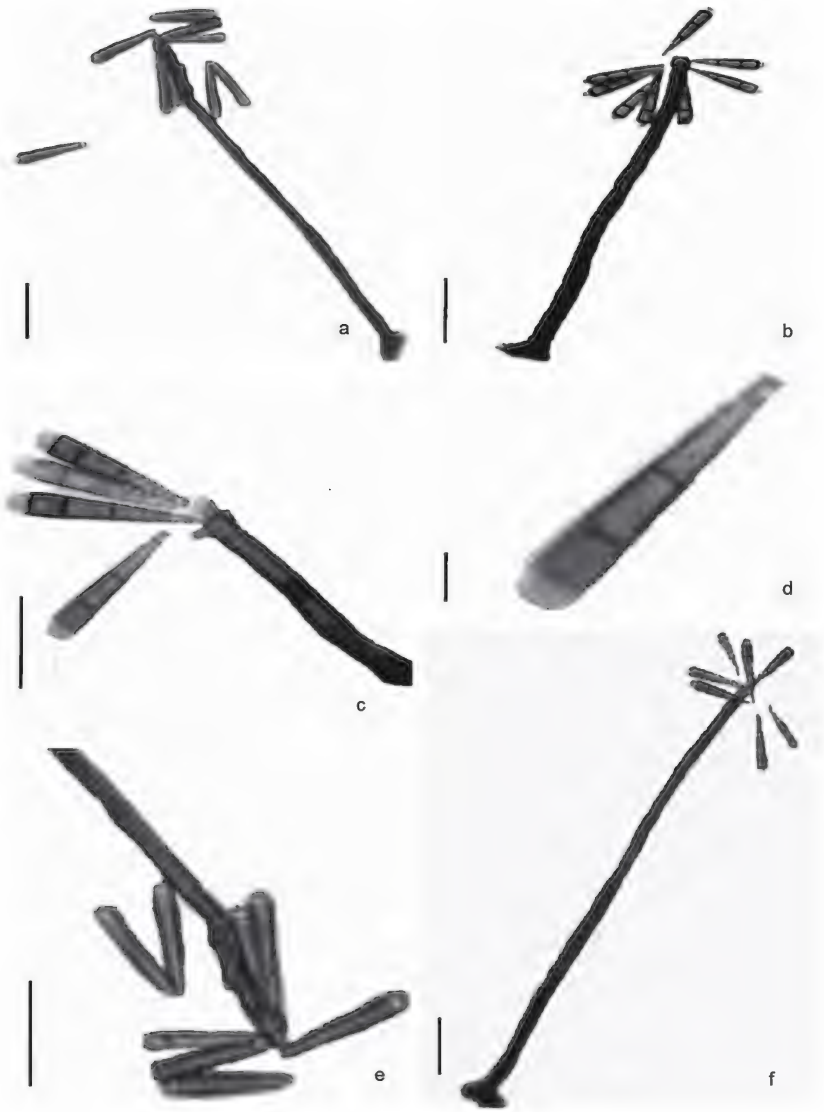

Plate 1. *Dactylaria pseudomanifesta:* A, B, F. Conidiophores (and conidia); C–E. Conidia. Scale bars: A, B, C, E, F = 15 µm; D = 3 µm.

Etymology: Latin *pseudomanifesta*, referring to the similarity with *Dactylaria manifesta*.

Colonies effuse, grayish, thin, without superficial mycelium and restricted immersed mycelium. Conidiophores simple, cylindrical, brown, septate, with

smooth walls, 55–188 × 5–6 µm. Conidiogenous cells terminal, integrated, denticulate 35–40 × 5–6 µm; denticles apical, in number of three to five, 1–2 µm long. Conidia obclavate to turbinate, 3-septate, light brown with a hyaline distal cell, 17–25 × 3.5–4 µm.

Additional specimen examined — **BRAZIL. Bahia**: municipality of Uruçuca, Parque Estadual Serra do Conduru, 14°23′07.7″S 39°04′43.6″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 12/7/2008, D.M.A. Magalhães s.n. (CEPEC 1417).

Comments — This species resembles *Dactylaria manifesta* R.F. Castañeda & W.B. Kendr., which differs by its smaller (14–20 × 3–3.5 µm) and totally colourless conidia (Castañeda-Ruiz & Kendrick 1991).

Beltraniella fertilis Heredia, R.M. Arias, M. Reyes & R.F. Castañeda, Fungal Diversity 11: 100 (2002). Plate 2a,b

Colonies hypophyllous, effuse. Immersed mycelium with setae upright, straight, single or clustered, thick walled, smooth to slightly warty, acute, dark brown, 90–240 × 3–6 µm, bulbous base. Conidiophores macronematous, setiform, single or in small groups, straight, septate, simple or branched at the apex, 97–337 × 40–8 µm. Separating cells unicellular, hyaline, ellipsoid, fusiform, 6–8 × 2.5–5 µm. Conidiogenous cells terminal polyblastic, integrated, pale brown. Conidia arising from conidiogenous cells or separating cells, rostrate, truncated at the base, smooth, olivaceous, with a hyaline transverse band above the equatorial zone, 15–23 × 5–8 µm.

Specimen examined — **BRAZIL. Bahia**: municipality of Una, Ecoparque, 15°10′44.6″S 39°02′06.4″W, on decomposing leaves of *Parinari alvimii*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1410).

Distribution — Described from Mexico in dead leaves of *Mangifera indica* (Heredia et al. 2002). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Heredia et al. (2002).

Chaetosphaeria innumera Berk. & Broome ex Tul. & C. Tul., Select. Fung. Carpol. 2: 252 (1863). Plate 2c

Colonies effuse, black. Perithecia unobserved. Conidiophores single or fasciculate, septate, dark brown, smooth, up to 200 µm long, 4–6 µm wide, often percurrent. Conidia clustered, ellipsoid to oblong, unicellular, olivaceous, 2.5–5 × 2–3 µm.

Specimen examined — **BRAZIL. Bahia**: municipality of Uruçuca, Parque Estadual Serra do Conduru, 14°23′08.4″S 39°04′37.9″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1463).

Distribution — Europe including Great Britain (Ellis 1976). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Ellis (1976). The teleomorphic phase was not observed.

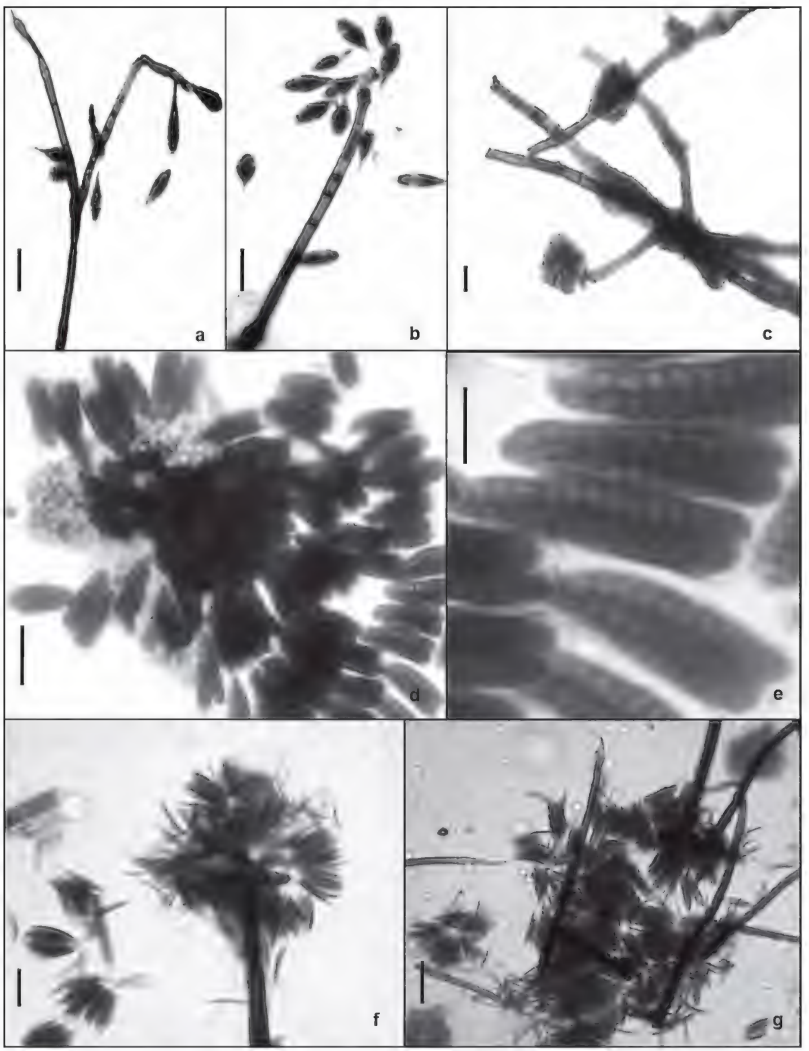

Plate 2. Fungi found in the Atlantic Forest of south Bahia — ***Beltraniella fertilis:*** A. Conidiophore branched at the apex; B. Conidia and conidiophores. ***Chaetosphaeria innumera:*** C. Fasciculate conidiophores and clustered conidia. ***Dictyosporium oblongum:*** D, E. Oblong conidia. ***Idriella acerosa:*** F. Terminal conidiogenous cells; G. Semilunar conidia. Scale bars: A, B = 15 μm; C = 5 μm; D, F, G = 10 μm; E = 30 μm.

Dictyosporium oblongum (Fuckel) S. Hughes, Can. J. Bot. 36: 762 (1958). Plate 2D,E

Colonies effuse. Conidia oblong or irregular, 32.5–37.5 × 10–12.5 μm, olivaceous, usually with 3–4 rows of cells of the same or different size, generally strongly constricted at the septa; cells 5 μm wide at 10–11 per row.

Specimen examined — **BRAZIL. Bahia:** municipality of Uruçuca, Serra do Condurú (PESC), 14°23′08.4″S 39°04′37.9″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1419).

Distribution — Recorded in Europe and North America (Ellis 1971; GBIF 2014). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Ellis (1971).

Idriella acerosa R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biology Series 35: 62 (1991). Plate 2f,g

Mycelium superficial composed of brown, branched, septate smooth, hyphae 15–20 µm wide. Conidiophores mononematic, unbranched, erect, straight, 4–6-septate, brown with almost colorless apex, 55–123 × 4–5 µm. Conidiogenous cells terminal sympodial with diminutive denticles almost colorless at the apex, 15–22 × 2.5–5 µm. Conidia falciform with acute ends, non-septate, dry, smooth and colorless, 10–15 × 1.5 µm.

Specimen examined — **BRAZIL. Bahia:** municipality of Uruçuca, Serra do Condurú (PESC), 14°23′08.4″S 39°04′37.9″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1421).

Distribution — Described from Cuba, on dead leaves of *Samanea saman* and *Nectandra coriacea* (Castañeda-Ruiz & Kendrick 1991). Mexico (GBIF 2014). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Castañeda-Ruiz & Kendrick (1991).

Inesiosporium longispirale (R.F. Castañeda) R.F. Castañeda & W. Gams, Nova Hedwigia 64: 486 (1997). Plate 3a

Mycelium superficial with septate, branched, smooth, brown to dark brown, hyphae 1–5 × 3–5 µm wide. Conidiophores undifferentiated, intercalated with conidiogenous cells, short, subulate, dark brown, monoblastic, 12–20 × 5–5.5 µm. Conidia acrogenous, single, coiled, twisted, usually attenuated and truncated at the base, multi-septate, olivaceous to pale brown, with 3–10 spirals, smooth walled, 70–l96 × 3–7 µm, spirals 50–65 µm × 18–36 µm, filaments 3–7 µm wide, olivaceous to pale brown, basal cells dark brown to brown.

Specimens examined — **BRAZIL. Bahia:** municipality of Una, Ecoparque, 15°10′01.7″S 39°03′14.2″W, on decomposing leaves of *Manilkara maxima*, 05/10/2008, D.M.A. Magalhães s.n. (CEPEC 1461); municipality of Itacaré, Reserva Capitão, 14°21′16.7″S 39°03′23.3″W, on decomposing leaves of *Manilkara maxima*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1556); 05/10/2008, D.M.A. Magalhães s.n. (CEPEC 1405); municipality of Uruçuca, Serra do Conduru, 14°23′07.7″S 39°04′43.6″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 05/10/2008, D.M.A. Magalhães s.n. (CEPEC 1462); 14°23′08.3″S 39°04′47.0″W, on decomposing leaves of *Manilkara maxima*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1553); 14°23′07.7″S 39°04′43.6″W,

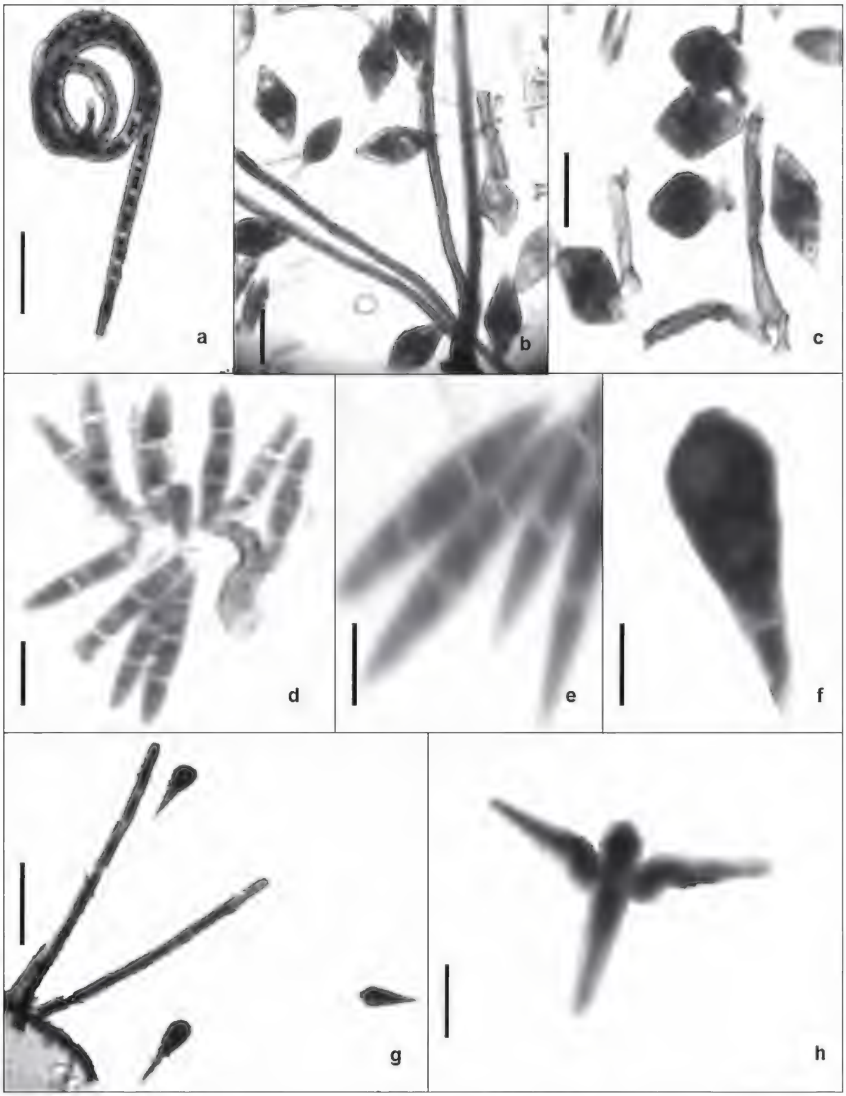

Plate 3. Fungi found in the Atlantic Forest of south Bahia — ***Inesiosporium longispirale:*** A. Coiled conidia. ***Pseudobeltrania macrospora:*** B, C. Conidia and conidiophores. ***Scolecobasidium tropicum:*** D. Conidia and conidiophore; E. Septate conidia. ***Sporidesmium coffeicola:*** F. Straight or curved conidiophores and conidia; G. Obpyriform septate conidia. ***Triscelophorus curviramifer:*** H. Obclaviform conidia. Scale bars: A, F = 10 μm; B, D, H = 15 μm; C = 30 μm; E, G = 5 μm.

05/10/2008, D.M.A. Magalhães s.n. (CEPEC 1503); 12/21/2007, D.M.A. Magalhães s.n. (CEPEC 1425); 14°23′06.6″S 39°04′44.7″W, on decomposing leaves of *Parinari alvimii*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1555).

Distribution — Found on dead leaves of *Calophyllum antillanum* and *Clusia rosea,* in Cuba (Castañeda-Ruiz & Gams 1997). First report for Brazil.

Comments — The specimens analyzed are similar to the description presented by Castañeda-Ruiz & Gams (1997).

Pseudobeltrania macrospora Heredia, R.M. Arias, M. Reyes & R.F. Castañeda, Fungal Diversity 11: 103 (2002). Plate 3B,C

Colonies epiphyllous, effuse. Mycelium immersed. Conidiophores macronematous, single or fasciculate, simple, septate, erect, brown, darker at the apex, 45–88 × 5–8 µm, with basal cell radiated-lobed, up to 20 µm in diameter. Conidiogenous cells polyblastic, terminal, cylindrical, pale brown, 15–30 × 6–7 µm. Conidia holoblastic, single, rhomboid, with acute base and apiculate apex, olivaceous, smooth, 30–45 × 15–20 µm, with hyaline equatorial band.

Specimen examined — **BRAZIL. Bahia:** municipality of Itacaré, Reserva Capitão, 14°21′17.6″S 39°03′23.4″W, on decomposing leaves of *Parinari alvimii*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1412).

Distribution — Mexico (Heredia et al. 2002). First report for Brazil.

Comments — The specimen fits the description presented by Heredia et al. (2002).

Scolecobasidium tropicum Matsush., Matsushima Mycological Memoirs 3: 15 (1983). Plate 3D,E

Colonies effuse. Hyphae superficial hyaline olivaceous, sparse. Conidiophores mononematous, macronematous, scattered, simple, cylindrical, 0–2-septate, brown, with denticulate ends elongating sympodially, 10–15 × 5 µm. Conidia fusiform, 2-septate, non-constricted at the septa, 17–20 × 3–4 µm, smooth, sub-hyaline.

Specimen examined — **BRAZIL. Bahia:** municipality of Una, at Ecoparque, 15°10′01.8″S 39°03′13.5″W, on decomposing leaves of *Manilkara maxima*, 05/10/2008, D.M.A. Magalhães s.n. (CEPEC 1418).

Distribution — Federated States of Micronesia, Canada, and Thailand, on dry petioles of *Cocos nucifera*. Republic of China (GBIF 2014). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Matsushima (1983).

Sporidesmium coffeicola M.B. Ellis, More Dematiaceous Hyphomycetes: 87 (1976). Plate 3F,G

Mycelium immersed. Conidiophores straight or curved, septate, brown, smooth, 75–110 × 3–5 µm, percurrent. Conidia obpyriform, 2–3-septate, 15–25 × 7–9 µm, 2–2.5 µm at the base, with the two lower cells dark brown and upper cells sub-hyaline.

Specimen examined — **BRAZIL. Bahia**: municipality of Uruçuca, at Serra do Condurú, 14°23′07.0″S 39°04′43.9″W, on decomposing leaves of *Harleyodendron unifoliolatum*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1409).

Distribution — Found in Iran (coffee leaves), Japan, and Mexico (GBIF 2014). First report for Brazil.

Comments — The specimen analyzed agrees with the description presented by Ellis (1976).

Triscelophorus curviramifer Matsush., Matsushima Mycological Memoirs 7: 70 (1993). Plate 3h

Conidia obclavate, 1–3-septate, 19–37 × 1.5–3.0 μm, with two obclavate branches, curved at the base, 0–1-septate, 9–22 × 1.4–2.5 μm.

Specimens examined — **BRAZIL. Bahia**: municipality of Una, Ecoparque, 15°10′44.6″S 39°03′06.4″W, on decomposing leaves of *Parinari alvimii*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1503); municipality of Uruçuca, Serra do Condurú (PESC), 14°23′08.3″S 39°04′47.0″W, on decomposing leaves of *Manilkara maxima*, 7/12/2008, D.M.A. Magalhães s.n. (CEPEC 1502).

Distribution — Peru on rotten leaves (Matsushima 1993), and Venezuela (Smits & Cressa 2005).

Comments — The specimens analyzed agree with the description presented by Delgado-Rodríguez et al. (2004).

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MYCOTAXON

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A new slender species of *Aureoboletus* from southern China

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Abstract — *Aureoboletus tenuis* sp. nov. is described from Guangxi Province, China. It is characterized by slender basidiomes with a glutinous, wrinkled, and light brown or reddish-brown to dark brown pileus. LSU sequence analysis confirms placement of the new species in *Aureoboletus*. A colour plate showing fruitbodies in situ and illustrations of microscopic elements are provided.

Key word — *Basidiomycota*, *Boletales*, taxonomy

Introduction

Aureoboletus was circumscribed by Pouzar (1957) (with *A. gentilis* (Quél.) Pouzar as type species) based on the following morphological features: small to medium sized basidiomes, an often viscid (but sometimes subtomentose) pileus, golden yellow or bright yellow (even when dried) tubes and pores, and a subequal or fusoid viscid to glutinous stipe that usually tapers to the base and is not distinctly reticulate or glandulose. Singer (1942), who had established *Xerocomus* sect. *Auripori* for *X. gentilis* [≡ *Aureoboletus gentilis*] and other species with golden yellow or bright olive-gold pores, a viscid pileus, and a gelatinous layer on the stipe (with or without a weak veil), later (1947) recombined it as *Pulveroboletus* sect. *Auripori*, which was to form the basis of Pouzar's new genus, *Aureoboletus*. Although not recognized as an independent genus by some mycologists (Corner 1972, Singer 1986, Šutara 2005), *Aureoboletus* has been accepted by others (Watling 1970, Pilát & Dermek 1974, Watling & Largent 1976, Alessio 1985, Dermek 1987, Li & Song 2002, Yang et

al. 2003, Kirk et al. 2008). Recent molecular studies on boletes now support *Aureoboletus* as an independent genus (Binder 1999, Binder & Hibbett 2006, Dentinger et al. 2010, Feng et al. 2012).

Of the twelve species and varieties placed within *Aureoboletus* (Klofac 2010), only two (*A. reticuloceps* M. Zang et al., *Aureoboletus thibetanus* (Pat.) Hongo & Nagas.) have been reported from China (Patouillard 1895, Zang et al. 1993, Ying & Zang 1994, Yang et al. 2003). However, one, *A. reticuloceps,* has been transferred to *Boletus* based on morphological and molecular evidence (Wang & Yao 2005, Dentinger et al. 2010, Feng et al. 2012). Recently, a new species, described here as *Aureoboletus tenuis*, was discovered in Guangxi Province, southern China.

Materials & methods

Specimens were photographed and annotated in the field and then dried in an electric drier. Type specimens were deposited in the Fungal Herbarium (GDGM) of Guangdong Institute of Microbiology, Guangzhou, China. Macroscopic description is based on fresh and dried specimens, field notes, and colour photographs. Colour notations follow Kornerup & Wanscher (1978). For descriptions of microscopic characters (including pileipellis and stipitipellis), tissue sections were revived and examined in 5% potassium hydroxide (KOH) or 1% Congo Red. Thirty basidiospores and 10 basidia were randomly selected from a mature specimen and measured in KOH; Q = spore length/width ratio; Q_m = mean ratio.

DNA was extracted from dried specimens using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The large subunit (nLSU) region was amplified by PCR, using primers LR0R and LR5 (Pinruan et al. 2010). The amplified products were determined by electrophoresis on 1% agarose gel against a known standard DNA marker and directly sequenced in Beijing Genomic Institute (BGI). The LSU sequence was submitted to GenBank. These and reference sequences from GenBank were used in phylogenetic analysis after being edited and aligned using Clustal 1.81 (Thompson et al. 1997) and MEGA5.1 (Tamura et al. 2011). The dataset was analyzed with maximum parsimony by PAUP* 4.0b10 (Swofford 2003) following He & Li (2013).

Taxonomy

Aureoboletus tenuis T.H. Li & Ming Zhang, **sp. nov.** Figs 1, 2

MycoBank MB 804773

Differs from *Aureoboletus auriporus* var. *novoguineensis* by its smaller fruitbodies and shorter basidiospores and from *A. thibetanus* by its wrinkled to shallowly reticulate pileus surface and the absence of appendiculate veil remnants.

Type: China, Guangxi Province, Maoʾershan National Nature Reserve, 25°50′N 110°19′E, 1387 m alt., on soil in a broadleaved forest dominated by *Fagaceae* (*Cyclobalanopsis* sp.), 16 July 2012, Ming Zhang (**Holotype**, GDGM 42601, GenBank KF534789).

Etymology: the specific epithet indicates the slender habit of the new species.

Fig 1. *Aureoboletus tenuis* (Holotype GDGM 42601). Basidiomes. Scale bar = 20 mm.

Basidiomes slender. Pileus 20–35 mm broad, hemispherical when young, becoming broadly convex to nearly plane in age, strongly glutinous when fresh, distinctly wrinkled to irregularly and shallowly reticulate, brown to reddish-brown (6D8–8D8, 8E8) at center and gradually paler outwards, deep orange (5A8–6A8), orange (5A7–6A7), orange-yellow (4A8–4A7), light yellow to pale yellow (3A4–4A4) at margin, with a slightly incurved edge when young. Context 3–4 mm thick at the centre of the pileus, thinner at pileus margin, soft, white to yellowish-white (1A1–1A2), more or less brown beneath the pileipellis, slightly changing pinkish-white (7A2–9A2) to pale red (7A3–9A3) when exposed. Tubes slightly depressed around stipe, light yellow to greenish-yellow (2A5, 2B5), 8–10 mm deep, unchanging on bruising. Pores 0.8–1 mm in diam., roundish to angular, somewhat compound and relatively larger around the stipe, concolorous with tubes. Stipe 40–70 × 3–7 mm, central, cylindrical, sometimes hollow and usually tapering towards the base, greyish-red to brownish-orange (7C4–7D4), smooth, without reticulation, sometimes longitudinally striate, gelatinous or strongly viscid, especially when young and wet, with a white (1A1) basal mycelium. Odor none. Taste mild.

Basidiospores (10–)11–12 × 4–5 μm, Q = (2.4–)2.5–2.8(–3), Q_m = 2.75 ± 0.29, n = 30, ellipsoid, smooth, yellowish to yellowish-brown in 5% KOH, thin-walled. Basidia 4-spored, 20–26 × 8–10 μm, clavate, yellowish-white to hyaline in 5% KOH, yellow to yellowish-brown in Melzer's. Sterigmata 2.5–3.5 μm

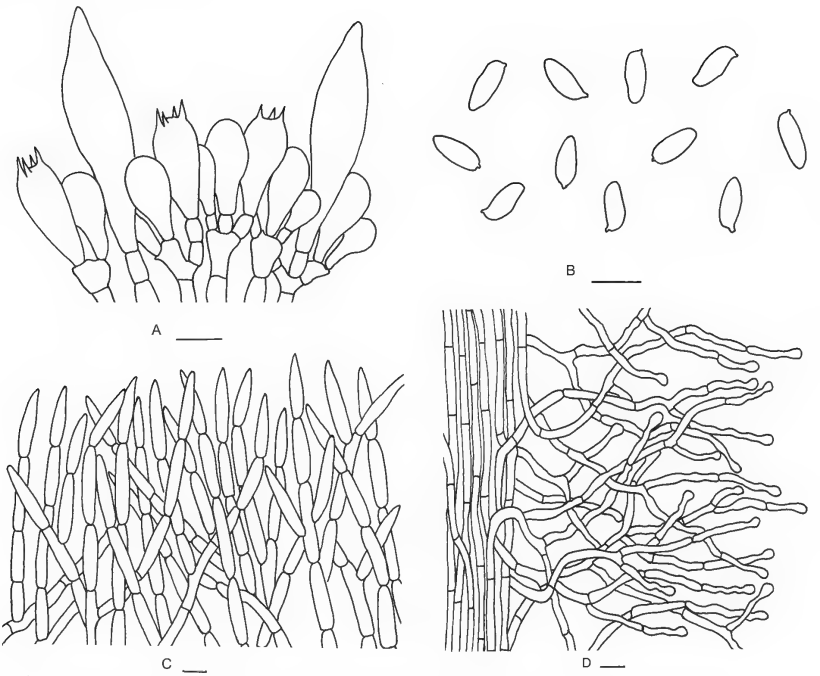

Fig. 2. Microscopic features of *Aureoboletus tenuis* (Holotype GDGM 42601). A. Basidia and pleurocystidia; B. Basidiospores; C. Pileipellis; D. Stipitipellis. Scale bars: A, B = 10 μm; C, D = 20 μm.

long. Pleurocystidia 40–53 × 11–15 μm, fusiform, thin-walled, yellowish-white to hyaline in 5% KOH. Cheilocystidia 42–52 × 11–15 μm, similar to pleurocystidia in shape and size. Hymenophoral trama subparallel to nearly bilateral, yellowish-white to hyaline in 5% KOH, composed of branching hyphae 6–9 μm wide, hardly to only slightly gelatinized. Pileipellis in young material usually an ixotrichodermium consisting of loosely and vertically arranged, frequently septate, thin-walled hyphae 6–12 μm in diam., tending to collapse in mature and dried specimens, yellowish white to hyaline in 5% KOH. Stipitipellis ixotrichodermial, producing branching hyphae 7–13 μm wide, with slightly swollen tips. Caulocystidia not observed. Clamp connections absent in all tissues.

Ecology & distribution — Solitary or scattered on soil in a broad-leaved forest dominated by *Fagaceae* (*Cyclobalanopsis* sp.). Known only from the type locality.

Comments — The diagnostic features of the new species include the small and slender basidiomes, glutinous and wrinkled pileus, cylindrical viscid

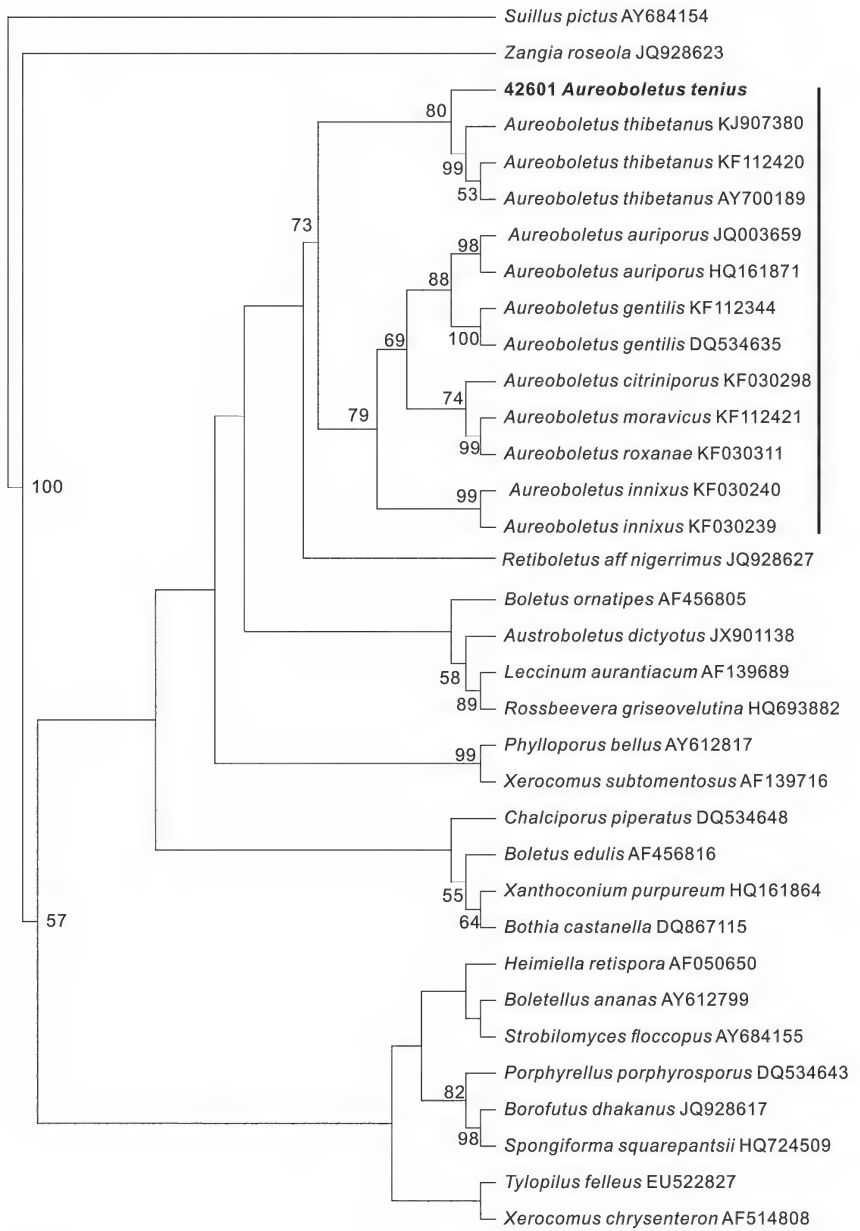

FIG. 3. The phylogenetic tree obtained from Maximum Parsimony analysis of LSU sequences of species of *Boletales*. *Suillus pictus* is selected as outgroup. Parsimony bootstrap values >50% are shown.

and sometimes hollow stipe, and relatively short basidiospores. This unique combination of characters easily separates *A. tenuis* from other *Aureoboletus* species.

Aureoboletus auriporus var. *novoguineensis* (Hongo) Klofac and *A. thibetanus* also have a glutinous and wrinkled pileus. However, *A. auriporus* var. *novoguineensis* differs by its larger and more robust fruitbodies, pale reddish-brown stipe, and longer basidiospores (11.5–15.5 µm; Hongo 1973, Klofac 2010), and *A. thibetanus* differs by its chestnut-brown, rusty-brown to pale brown, more distinctly reticulate-alveolate pileus that is ornamented by strongly gelatinized veil remnants hanging at margin, longer basidiospores (9.0–15.0 × 4.0–5.5 µm), and thin-walled cystidia with a refractive substance on the surface (Patouillard 1895, Yang et al. 2003, Klofac 2010).

Aureoboletus tenuis is easily differentiated from similarly coloured taxa such as *A. flaviporus* (Earle) Klofac and *A. roxanae* (Frost) Klofac, as *A. flaviporus* exhibits more robust fruitbodies and larger basidiospores (11–18 × 4–6 µm; Earle 1904, Both 1993, Bessette et al. 2000, Klofac 2010) while *A. roxanae* has a dry and broader pileus (≤90 mm) and sturdier stipe (≤70 × 16 mm; Frost 1874, Smith & Thiers 1971, Bessette et al. 2000, Klofac 2010).

The phylogenetic tree (Fig. 3) clusters all sampled *Aureoboletus* species in a clade with 73% bootstrap support and *A. tenuis* and *A. thibetanus* in a subclade with a 80% bootstrap value. This well-supported lineage indicates that the new taxon *A. tenuis* is sister to *A. thibetanus*, but the blast result with nrLSU sequence shows that the two species share only 97% max identity. Thus, *A. tenuis* is phylogenetically and morphologically distinct from all the sampled species.

Acknowledgments

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Nomenclatural revision of four *Peziza* species

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Abstract — Three replacement names and a new combination are published for four *Peziza* species whose current names in *Peziza* are illegitimate later homonyms: *Peziza delilei* nom. nov. (≡ *P. viridifusca* Delile ex De Seynes), *Peziza elaeocarpa* comb. nov. (≡ *P. olivacea* Quél.), *Peziza pauli* nom. nov. (≡ *P. martinii* Donadini), and *Peziza queletii* nom. nov. (≡ *P. ampelina* Quél.).

Key words — *Pezizaceae*, *Pezizales*, nomina nova

Some species of European *Peziza* Dill. ex Fr. : Fr. have no legitimate names in *Peziza* and are known currently by illegitimate names (later homonyms; McNeill et al. 2012, Article 53.1). This work resolves these nomenclatural irregularities.

Peziza delilei Medardi, Lantieri & Cacialli, **nom. nov.**

MycoBank MB808495

≡ *Peziza viridifusca* Delile ex De Seynes, in De Seynes, Veg. Inf. 3: 83. 1886, nom. illegit., non *Peziza viridifusca* Fuckel 1870.

≡ *Humaria viridifusca* Sacc., Syll. Fung. 8: 149. 1889.

≡ *Galactinia viridifusca* (Sacc.) Boud., Hist. Class. Discom. Eur.: 48. 1907.

Etymology: after Alire Raffenau Delile, who discovered the species.

Peziza elaeocarpa (Sacc.) Medardi, Lantieri & Cacialli, **comb. nov.**

MycoBank MB808494

≡ *Peziza olivacea* Quél., Bull. Soc. Bot. Fr. 25(4): 291. 1879 ["1878"], nom. illegit., non *Peziza olivacea* Batsch 1783.

≡ *Humaria elaeocarpa* Sacc., Syll. Fung. 8: 148. 1889.

Peziza pauli Medardi, Lantieri & Cacialli, **nom. nov.**

MycoBank MB808493

≡ *Peziza martinii* Donadini, Bull. Soc. Linn. Provence 35: 171. 1985 ["1984"], as "*martini*", nom. illegit., non *Peziza martinii* Pers. : Fr. 1822.

Etymology: from *Paulus*, the Latinized baptismal name of Paul Martin, to whom the species was originally dedicated.

Peziza queletii Medardi, Lantieri & Cacialli, **nom. nov.**

MycoBank MB808492

≡ *Peziza ampelina* Quél., Grevillea 8(47): 116. 1880, nom. illegit., non *Peziza ampelina* Pass. 1874.

≡ *Plicaria ampelina* Rehm, Rabenh. Krypt.-Fl., Ed. 2, 1(3): 1003. 1894.

≡ *Galactinia ampelina* (Rehm) Boud., Hist. Class. Discom. Eur.: 47. 1907.

Etymology: after Lucien Quélet, who discovered the species.

Comments: We do not accept the synonymy of *P. ampelina* Quél. with *P. arenaria* Osbeck 1762 (as reported by Species Fungorum, http://www.speciesfungorum.org/GSD/GSDspecies.asp?RecordID=148680); the few characters of Osbeck's original diagnosis of *P. arenaria* delineate a brown, cup-shaped, sabulicolous species with a split margin, not a typical violaceous, saucer-shaped fungus, which is *P. ampelina* of Quélet.

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Volume 128, pp. 205 *April–June 2014*

Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce the posting of a new species distribution list by Calaça, Silva, & Xavier-Santos, who present the coprophilous and other dung-related fungi recorded in Brazil. This brings to 112 the number of free access mycobiotas now available on the Mycotaxon website: http://www.mycotaxon.com/resources/weblists.html

South America

Brazil

Francisco Junior Simões Calaça, Nathan Carvalho da Silva, & Solange Xavier-Santos. A checklist of coprophilous fungi and other fungi recorded on dung from Brazil. 22 p.

Abstract — A review of the literature published between 1919 (the earliest known record) and 2013 has made it possible to confirm the occurrence of 209 species of coprophilous fungi (*sensu lato*) in Brazil, which are distributed in 259 records in 12 states of the Federation, with Pernambuco being the State most represented. The phylum most found was *Ascomycota* (117 species), followed by *Zygomycota* (54), *Basidiomycota* (25), *Myxomycota* (11), *Oomycota* (1) and *Proteobacteria* (1).

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≡ *Peziza martinii* Donadini 1985, nom. illegit.

Peziza queletii Medardi, Lantieri & Cacialli, p. 204
≡ *Peziza ampelina* Quél. 1880, nom. illegit.

Puccinia caricis-araliae Kakish. & Q. Wang (neotypified, epitypified), p. 75
≡ *Aecidium araliae* Sawada ex S. Ito & Muray. (1943)
non *Puccinia araliae* Ellis & Everh. (1891)

Ravenelia costae Salazar-Yepes & A.A. Carvalho, p. 20

Sphaceloma heliciae H.Y. Yang & Z.Y. Zhang, p. 41
Thalloloma ochroleucum Z.F. Jia & Kalb, p. 114
Tylopilus sultanii S. Sarwar, Khalid & Niazi, p. 3
Xiuguozhangia K. Zhang, R.F. Castañeda, Jian Ma & L.G Ma, p. 132
Xiuguozhangia appendiculata (Bhat & W. B. Kendr.,) K. Zhang & R.F. Castañeda, p. 133
Xiuguozhangia indica (Sharath, Sureshk., Kunwar & Manohar.) K. Zhang & R.F. Castañeda, p.134
Xiuguozhangia punicae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, p. 134
Xiuguozhangia rhaphidophorae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, p. 134
Xiuguozhangia rosae (K. Zhang & X.G. Zhang) K. Zhang & R.F. Castañeda, p. 133
Xylaria hongkongensis A.M.C. Tang, R.Y.C. Lam, M.W.K. Leung, p. 38